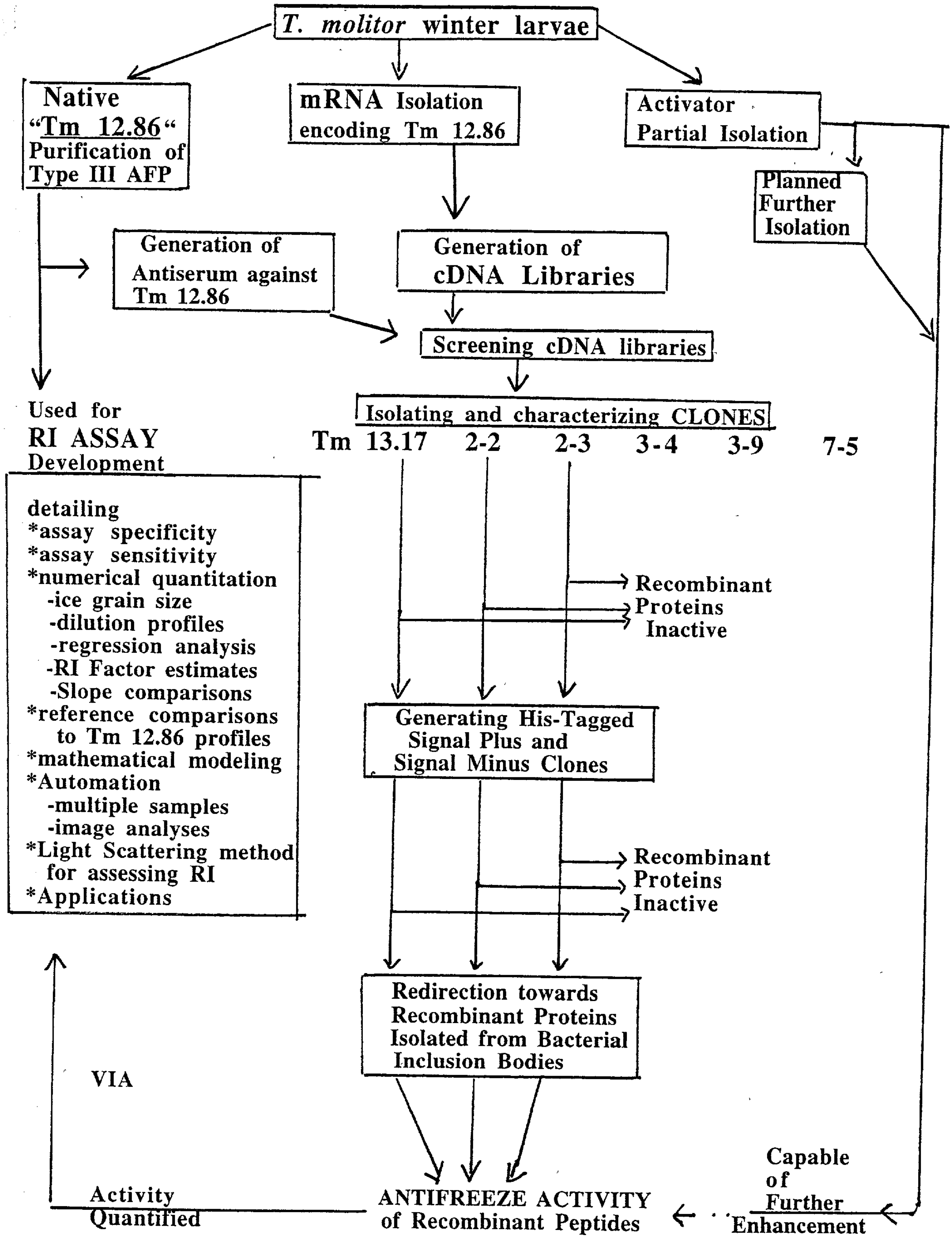

T. molitor winter larvae
Native "Tm 12.86" Purification of Type III AFP
mRNA Isolation encoding Tm 12.86
Activator Partial Isolation
Planned Further Isolation
Generation of Antiserum against Tm 12.86
Generation of cDNA Libraries
Screening cDNA libraries
Isolating and characterizing CLONES
Tm 13.17 2-2 2-3 3-4 3-9 7-5
Used for RI ASSAY Development
detailing
*assay specificity
*assay sensitivity
*numerical quantitation
-ice grain size
-dilution profiles
-regression analysis
-RI Factor estimates
-Slope comparisons
*reference comparisons to Tm 12.86 profiles
*mathematical modeling
*Automation
-multiple samples
-image analyses
*Light Scattering method for assessing RI
*Applications
Recombinant Proteins Inactive
Generating His-Tagged Signal Plus and Signal Minus Clones
Recombinant Proteins Inactive
Redirection towards Recombinant Proteins Isolated from Bacterial Inclusion Bodies
VIA
Activity Quantified
ANTIFREEZE ACTIVITY of Recombinant Peptides
Capable of Further Enhancement

FIG 1.0

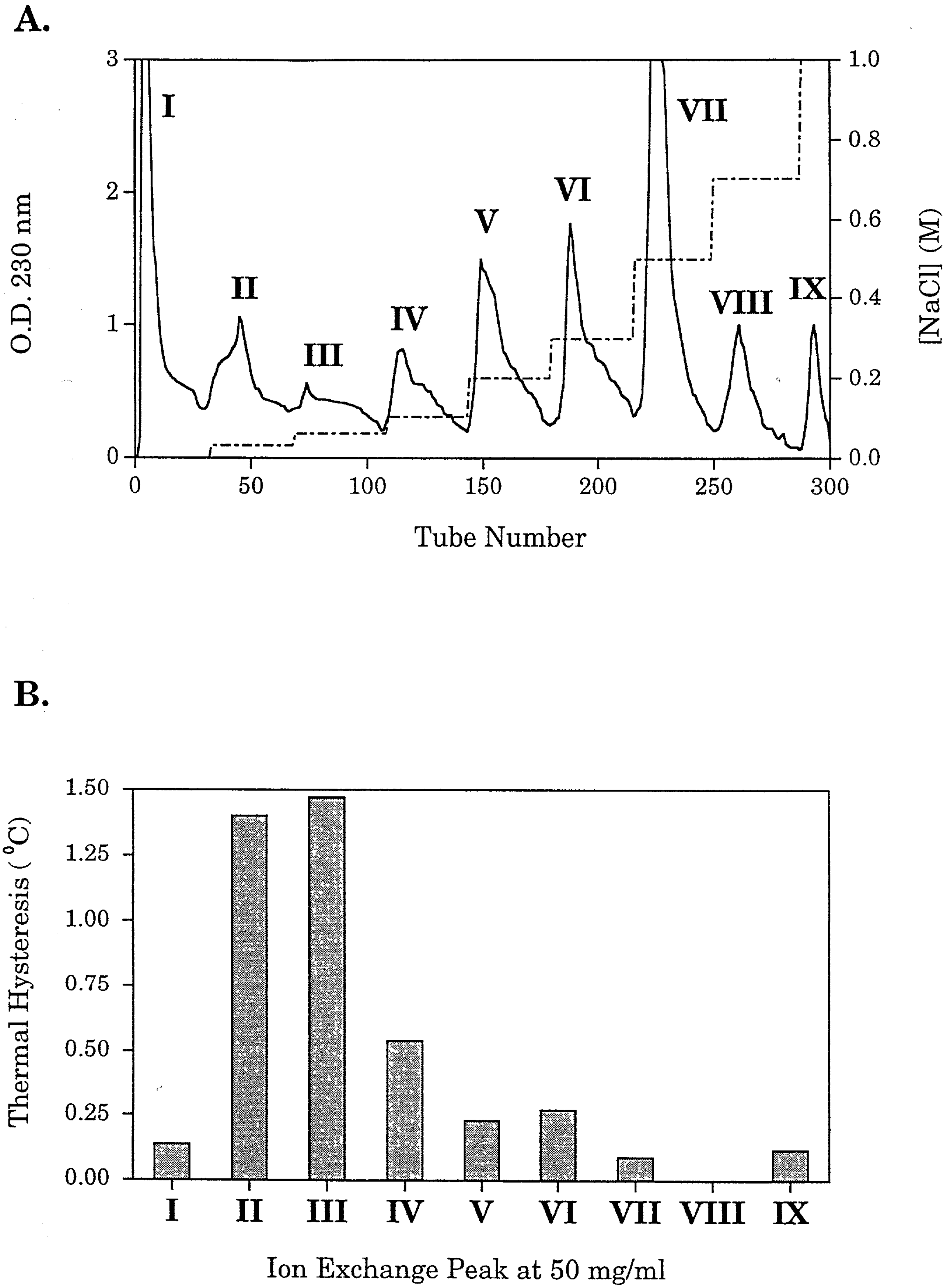

A.
I
II
III
IV
V
VI
VII
VIII
IX
O.D. 230 nm
[NaCl] (M)
Tube Number
B.
Thermal Hysteresis (°C)
I
II
III
IV
V
VI
VII
VIII
IX
Ion Exchange Peak at 50 mg/ml

FIG 1.1

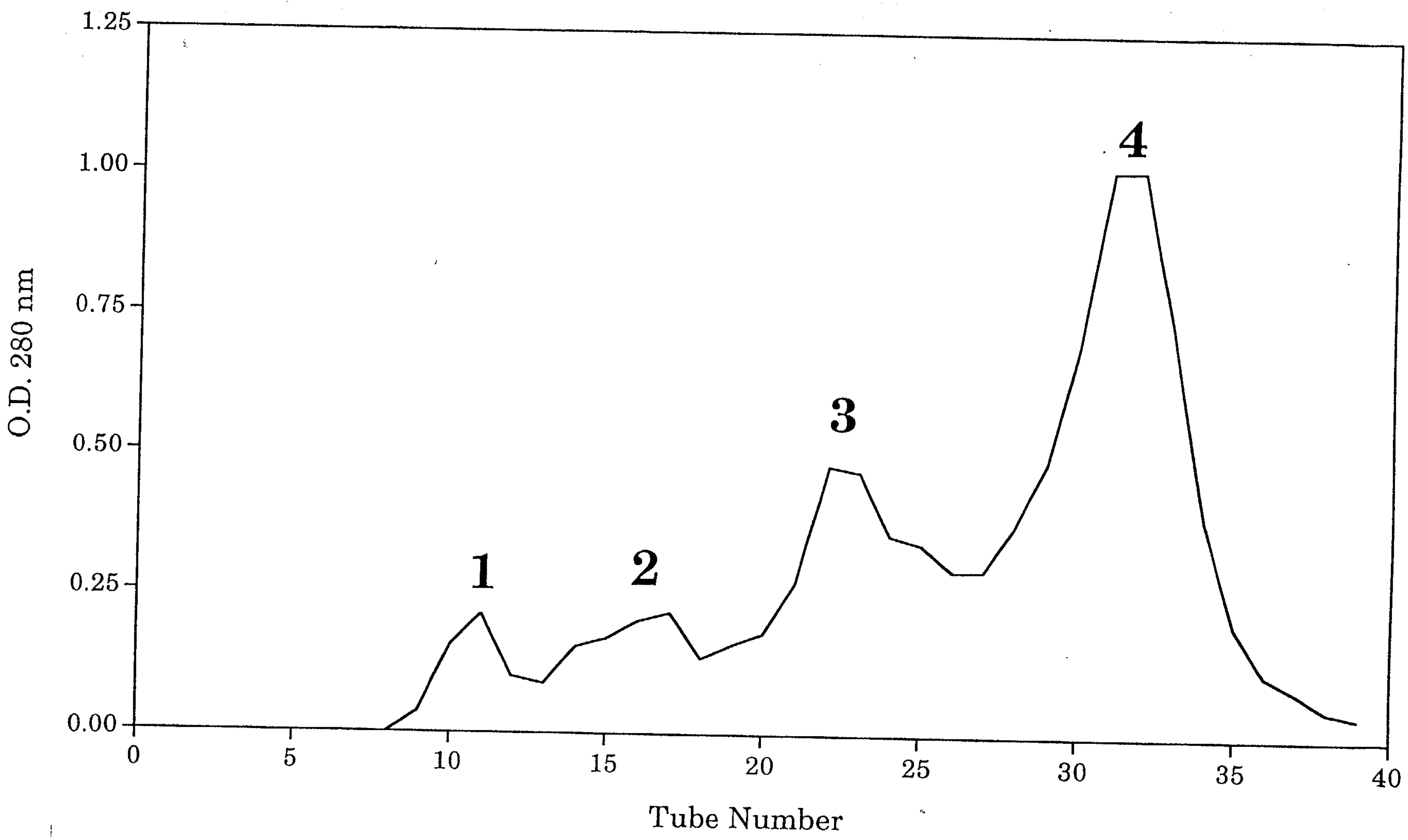

1.25
1.00
0.75
0.50
0.25
0.00
O.D. 280 nm
0
5
10
15
20
25
30
35
40
Tube Number
1
2
3
4

FIG 1.2

FIG 1.3

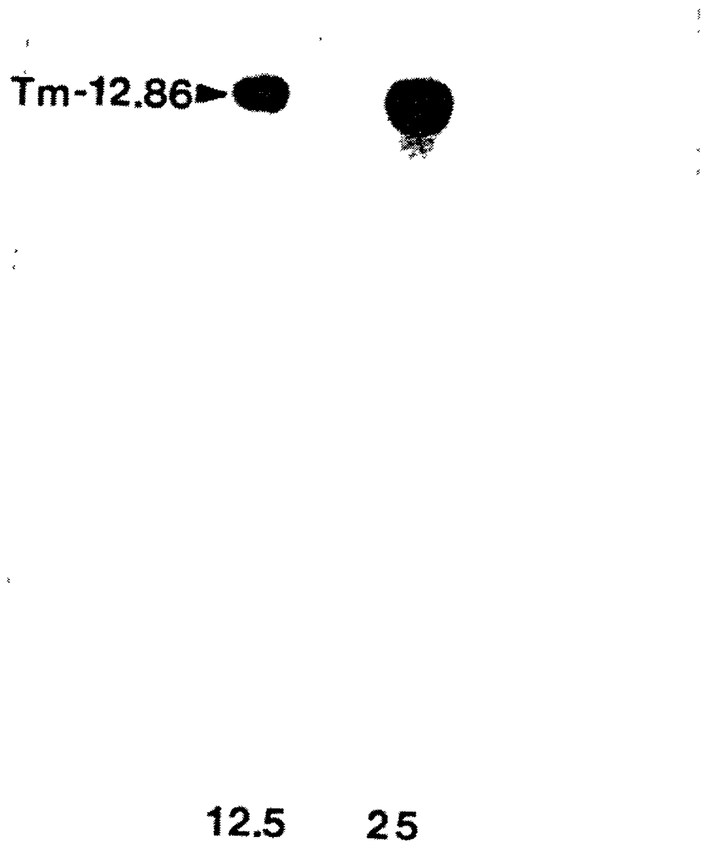


FIG 1.4

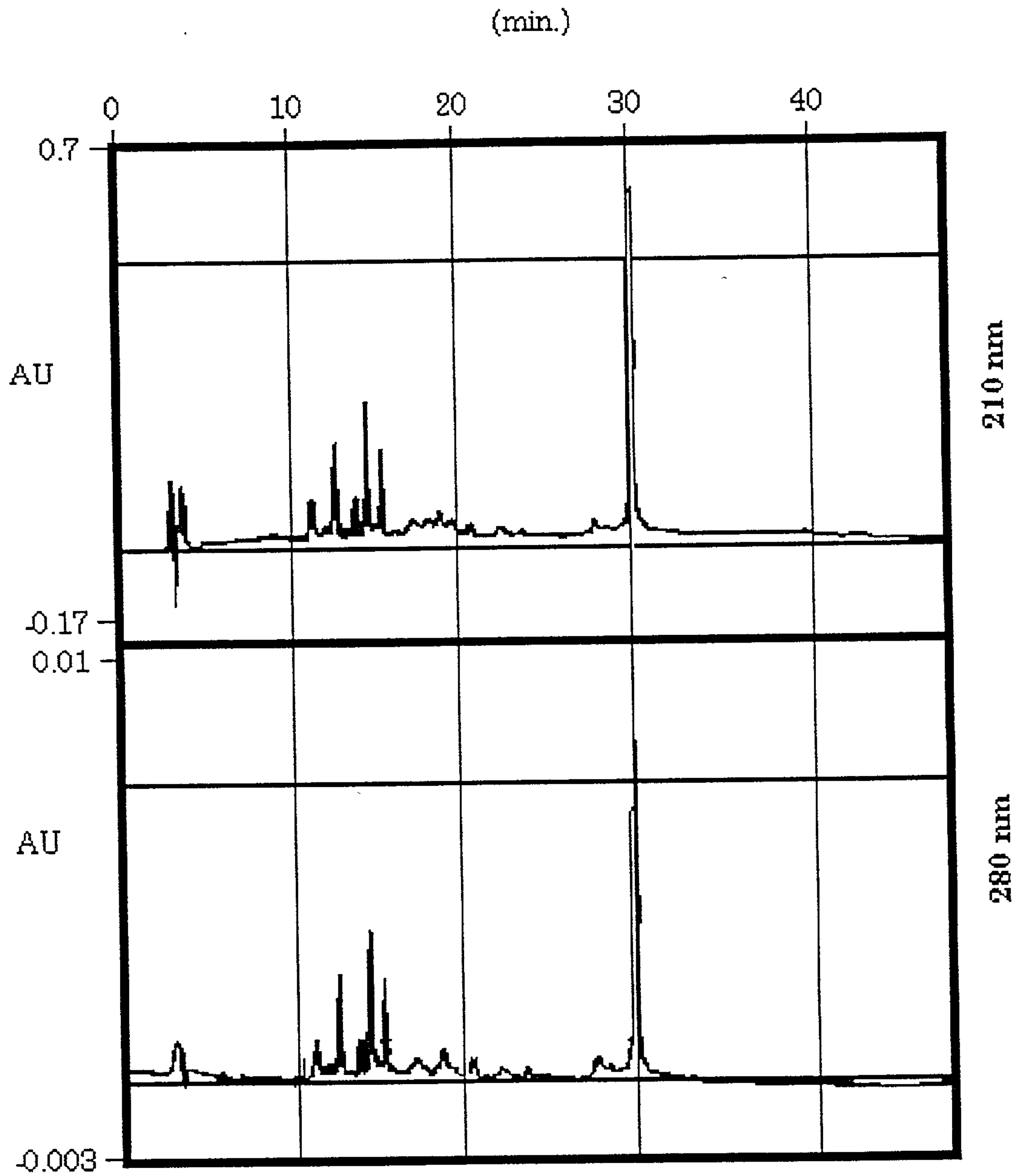
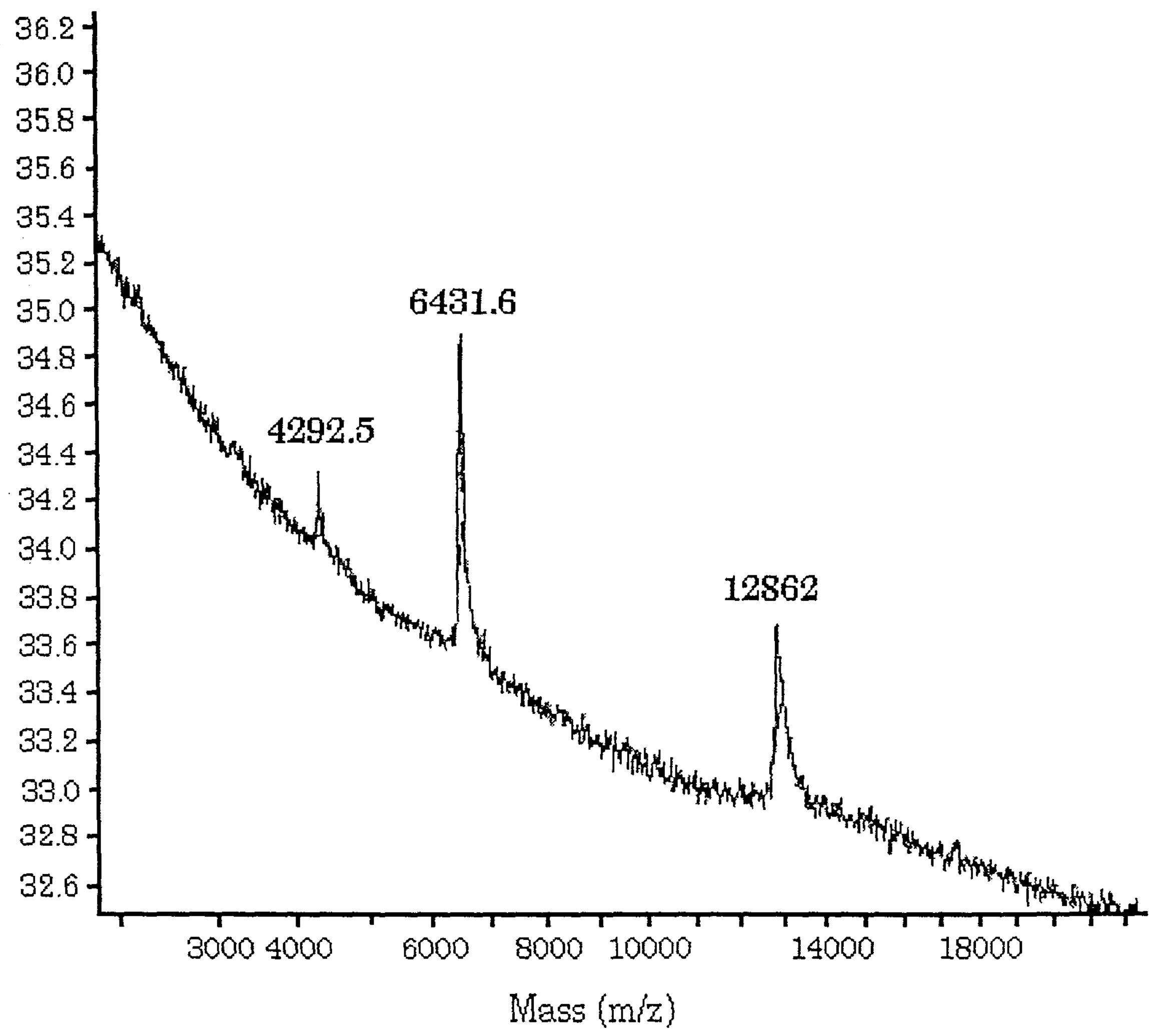


FIG 1.5



36.2
36.0
35.8
35.6
35.4
35.2
35.0
34.8
34.6
34.4
34.2
34.0
33.8
33.6
33.4
33.2
33.0
32.8
32.6
6431.6
4292.5
12862
3000 4000
6000
8000
10000
14000
18000
Mass (m/z)

FIG 1.6

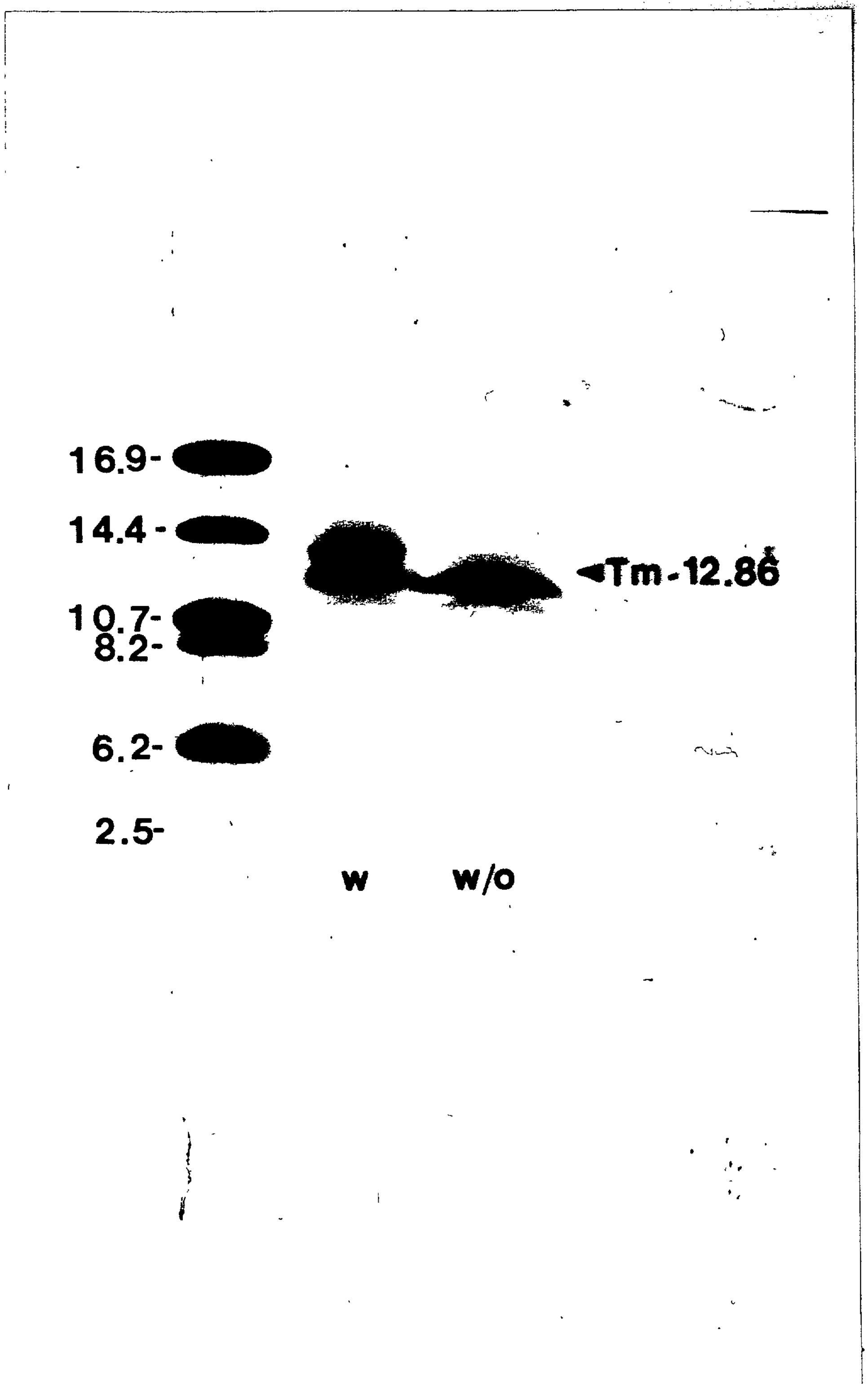


FIG 1.7

NH_2-L-T-D-E-Q-I-Q-K-R-N-K-I-S-K-E-?-Q-Q-V

L	T	D	E	Q	I	Q	K	R	N	K	I	S	K	E	?	Q	Q	V
Leu	Thr	Asp	Glu	Gln	Ile	Gln	Lys	Arg	Asn	Lys	Ile	Ser	Lys	Glu		Gln	Gln	Val

FIG. 1.8

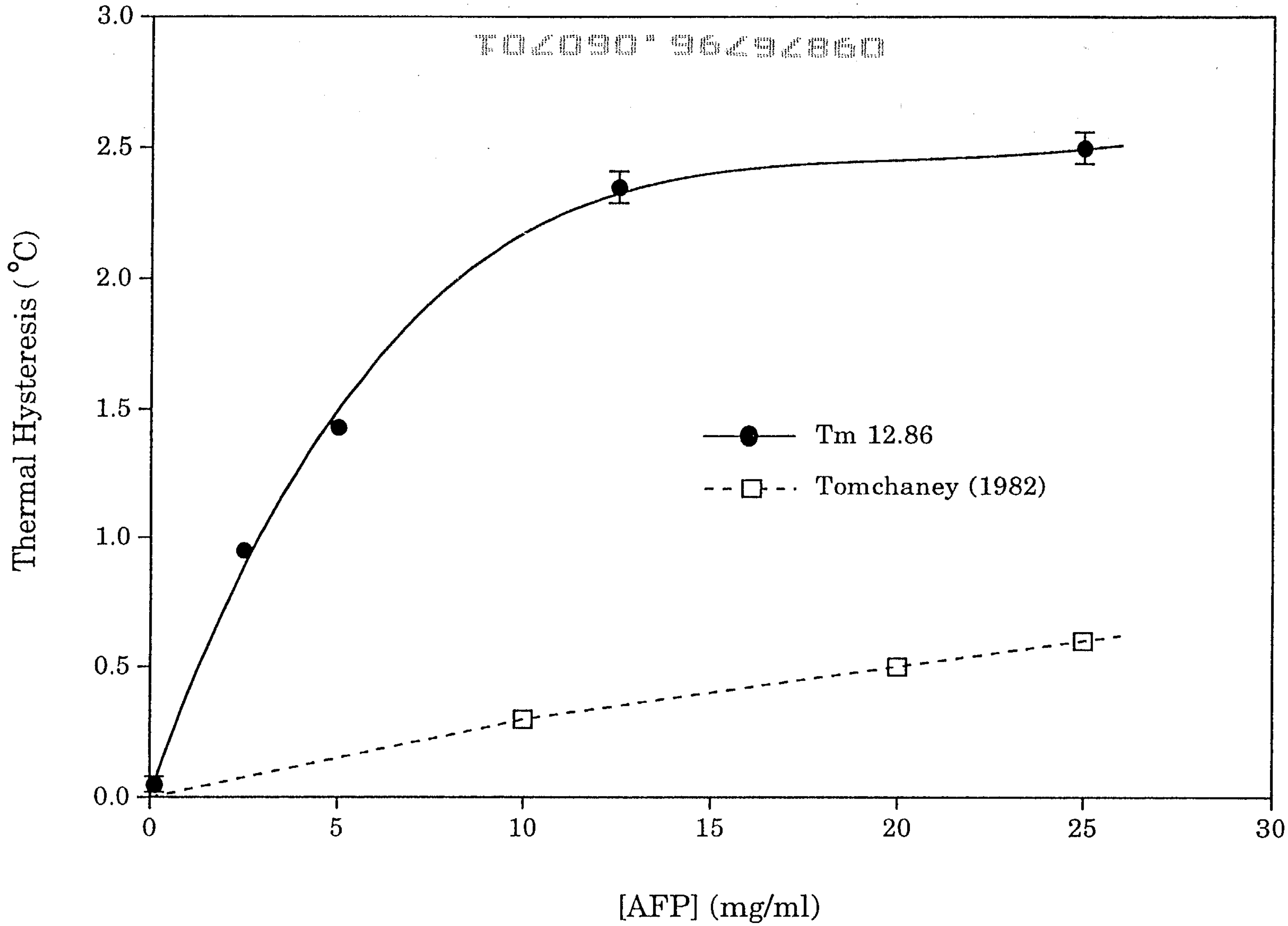

Thermal Hysteresis (°C)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
[AFP] (mg/ml)
Tm 12.86
Tomchaney (1982)

FIG 1.9

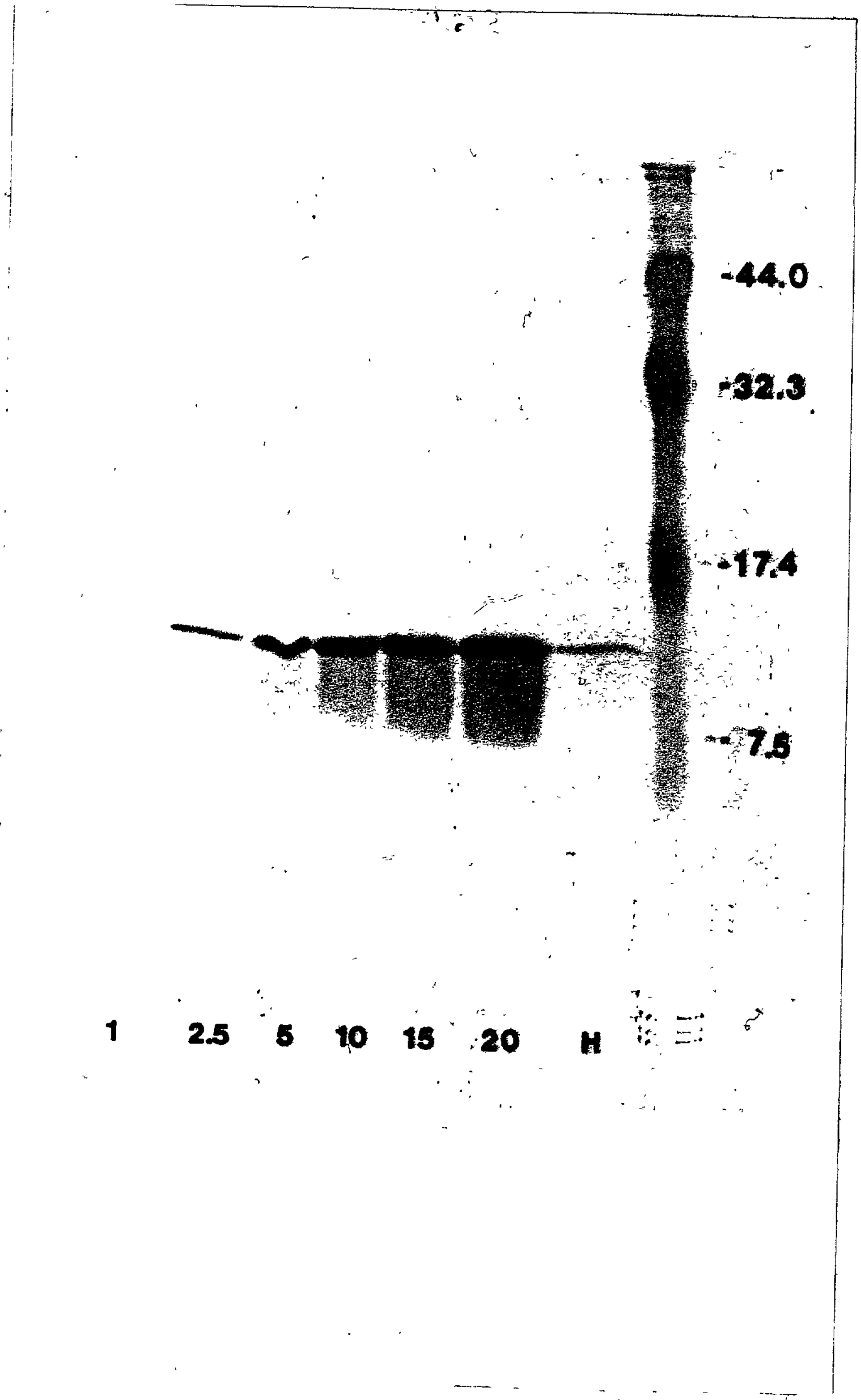


FIG 1.10

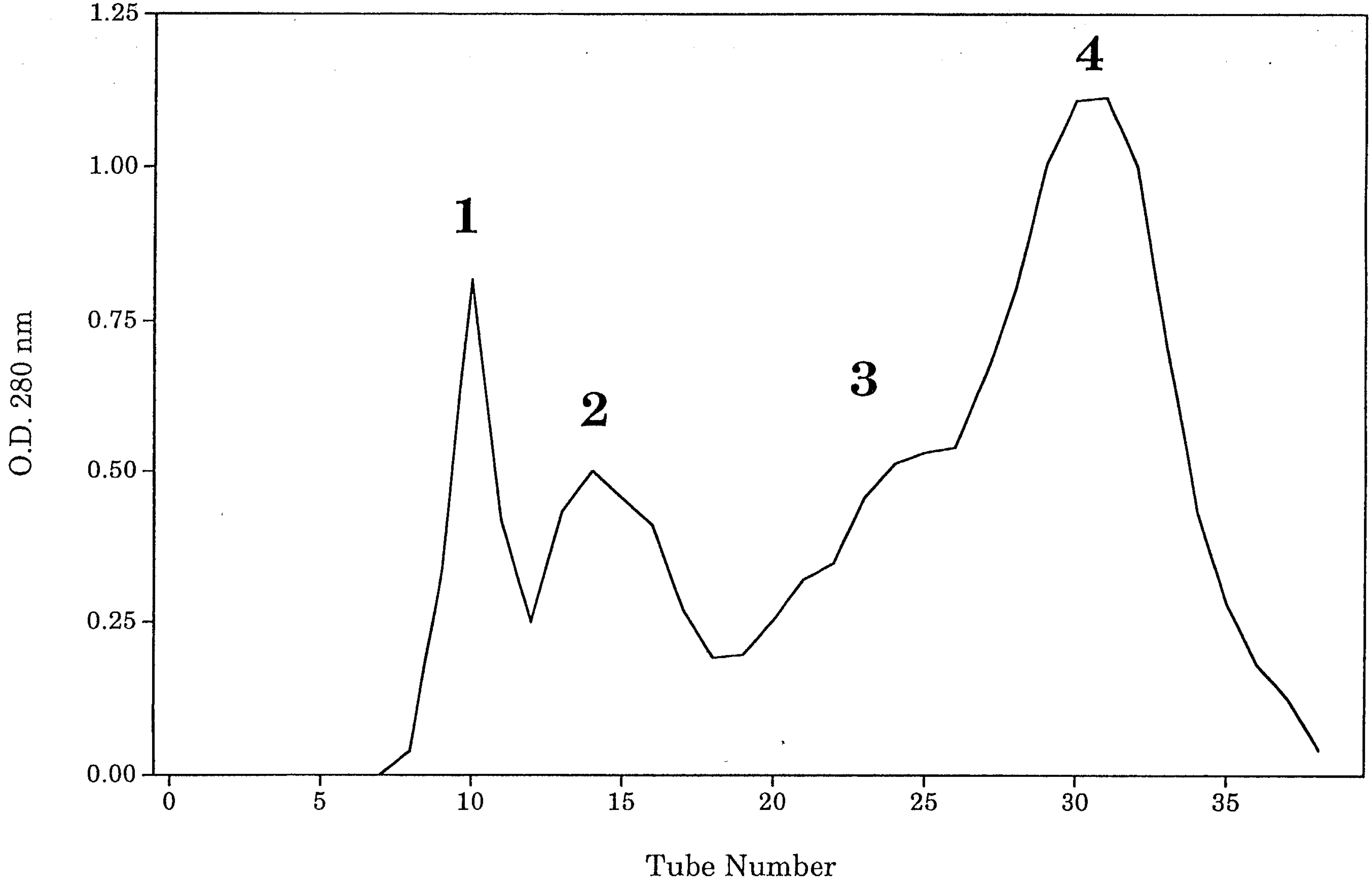

1.25
1.00
0.75
0.50
0.25
0.00
O.D. 280 nm
0
5
10
15
20
25
30
35
Tube Number
1
2
3
4

FIG 1.11

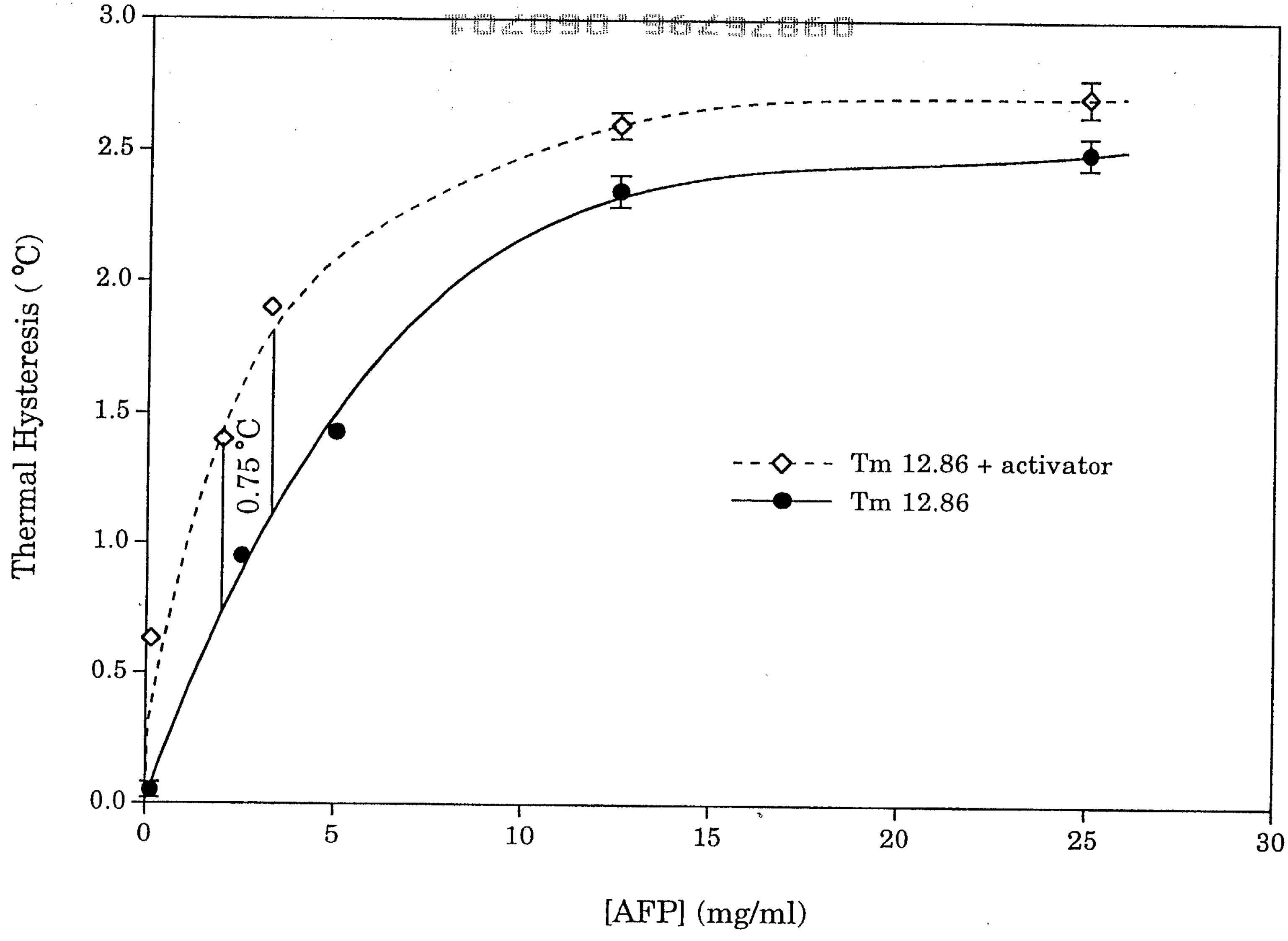
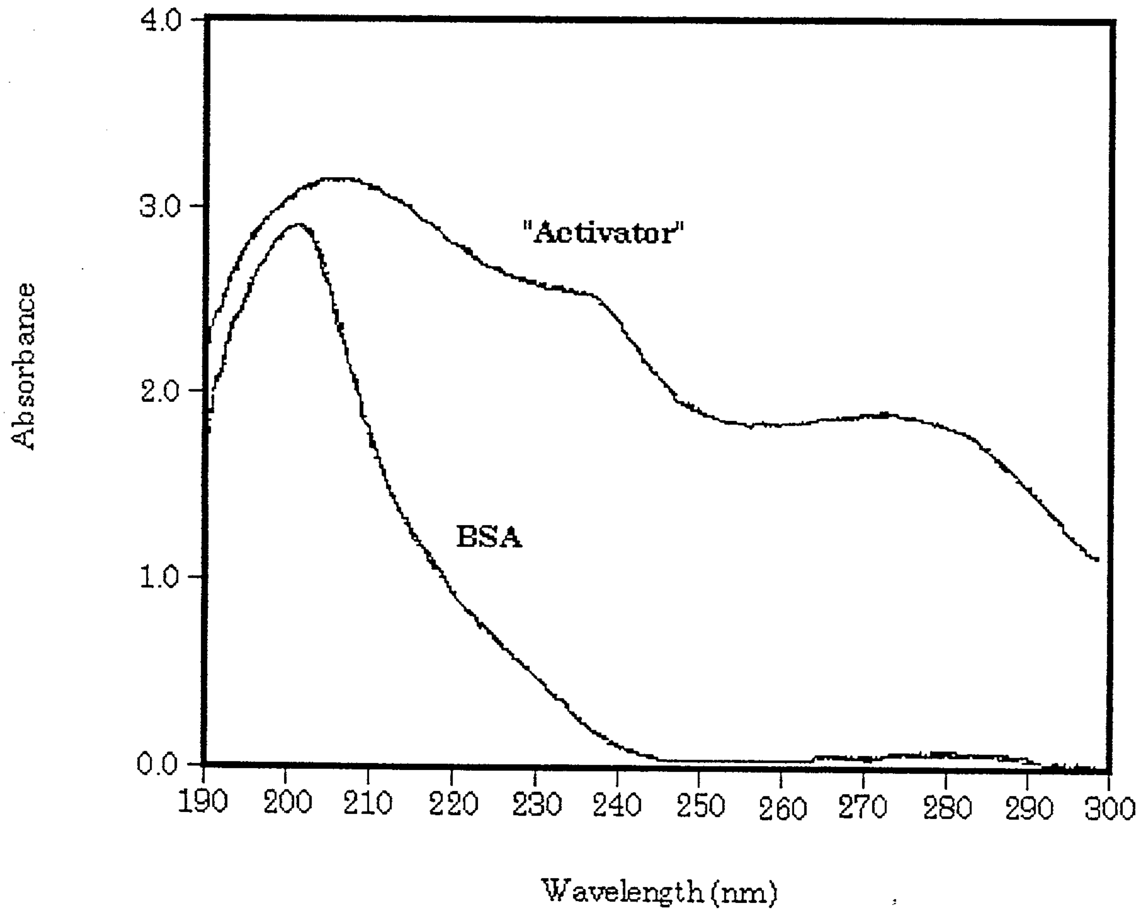

3.0
2.5
2.0
1.5
1.0
0.5
0.0
Thermal Hysteresis (°C)
0
5
10
15
20
25
30
[AFP] (mg/ml)
0.75 °C
Tm 12.86 + activator
Tm 12.86

FIG 1.12



4.0
3.0
2.0
1.0
0.0
Absorbance
"Activator"
BSA
190
200
210
220
230
240
250
260
270
280
290
300
Wavelength (nm)

FIG 1.13

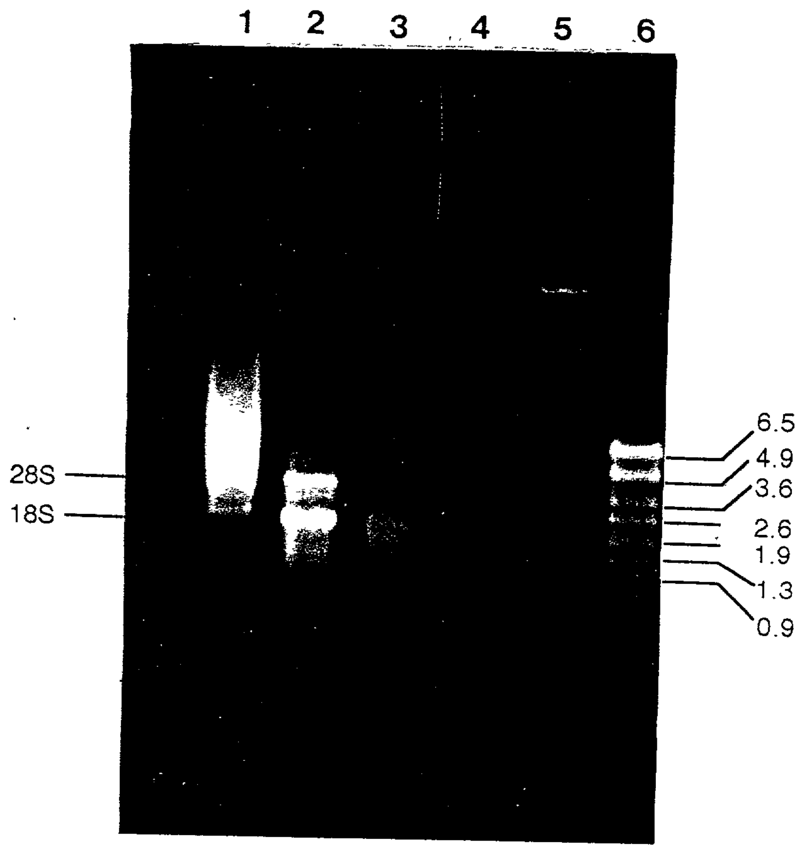


FIG 2.0

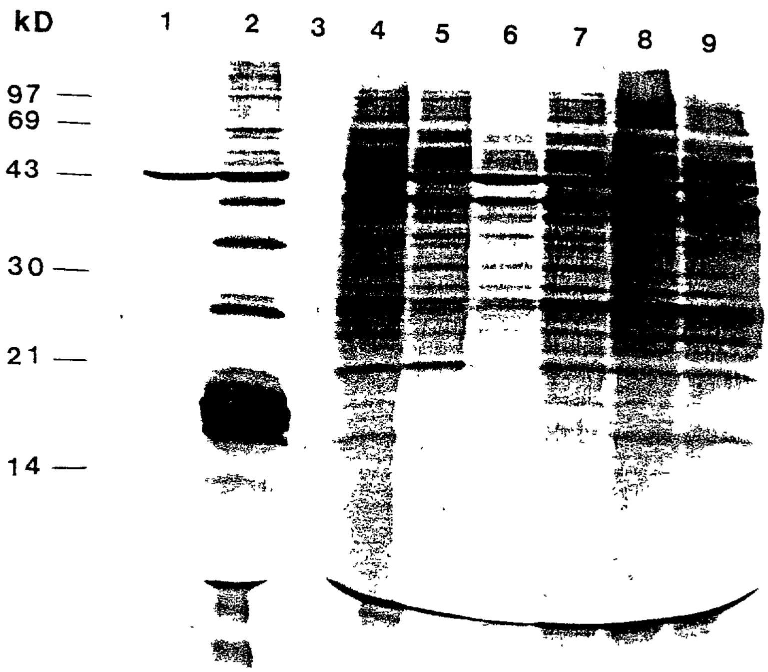


FIG 2.1

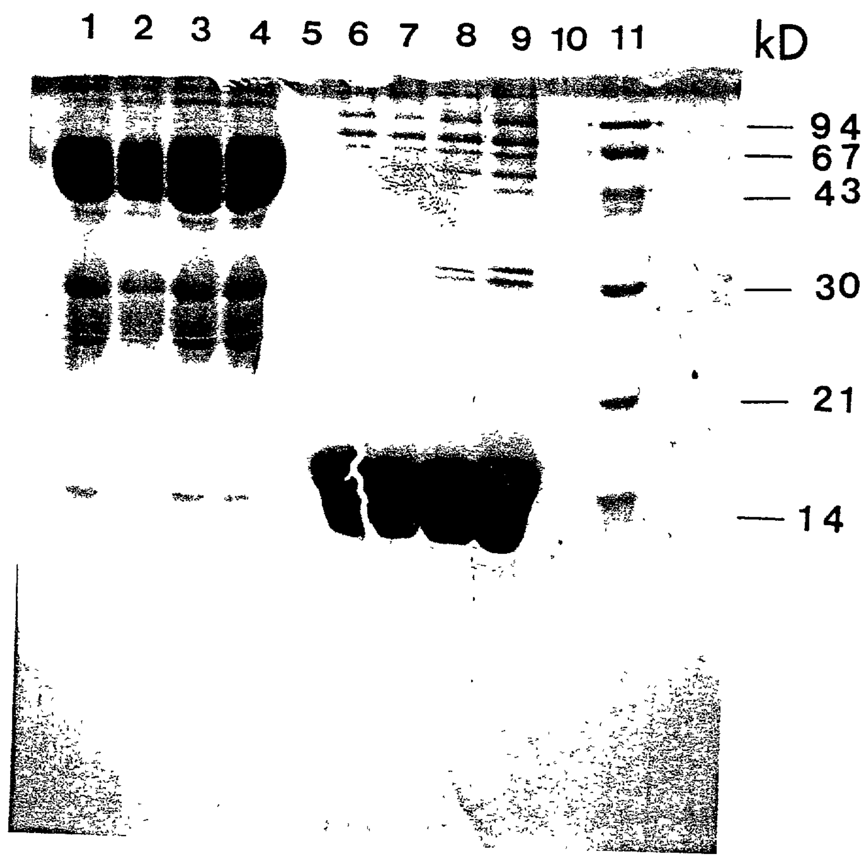


FIG 2.2

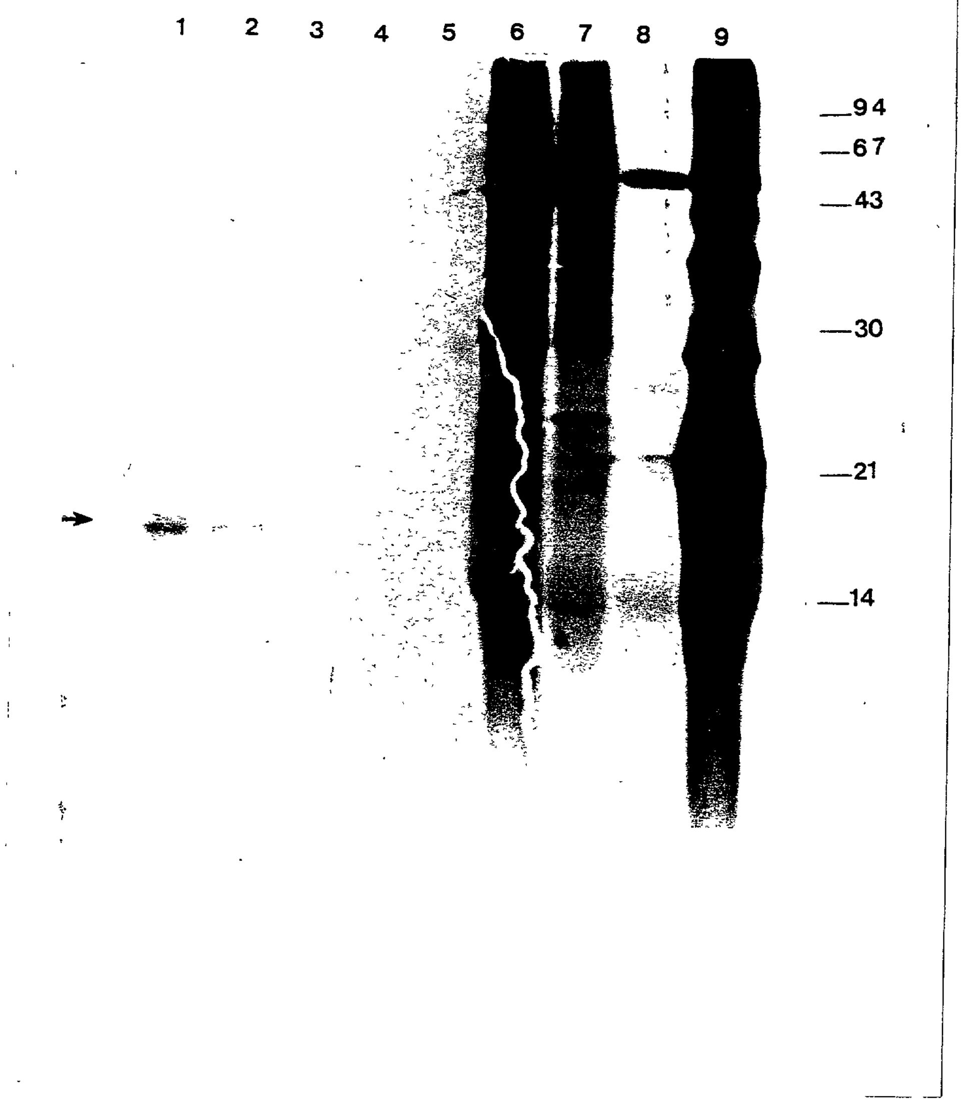


FIG 2.3

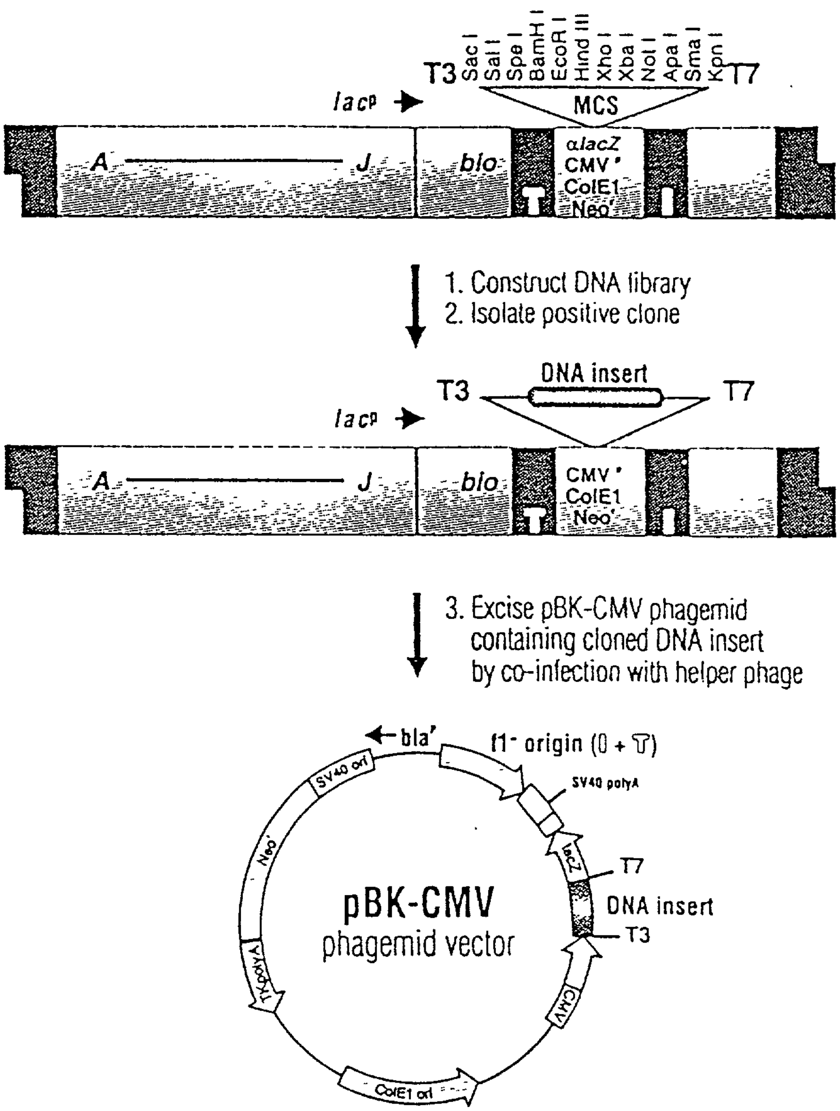

T3
Sac I
Sal I
Spe I
BamH I
EcoR I
Hind III
Xho I
Xba I
Not I
Apa I
Sma I
Kpn I
T7
lacp
MCS
A
J
bla
αlacZ
CMV'
ColE1
Neo'
1. Construct DNA library
2. Isolate positive clone
DNA insert
T3
T7
lacp
A
J
bla
CMV'
ColE1
Neo'
3. Excise pBK-CMV phagemid
containing cloned DNA insert
by co-infection with helper phage
bla'
f1⁻ origin
SV40 ori
SV40 polyA
lacZ
T7
DNA insert
T3
Neo'
pBK-CMV
phagemid vector
TK polyA
CMV
ColE1 ori

FIG. 2.4 a

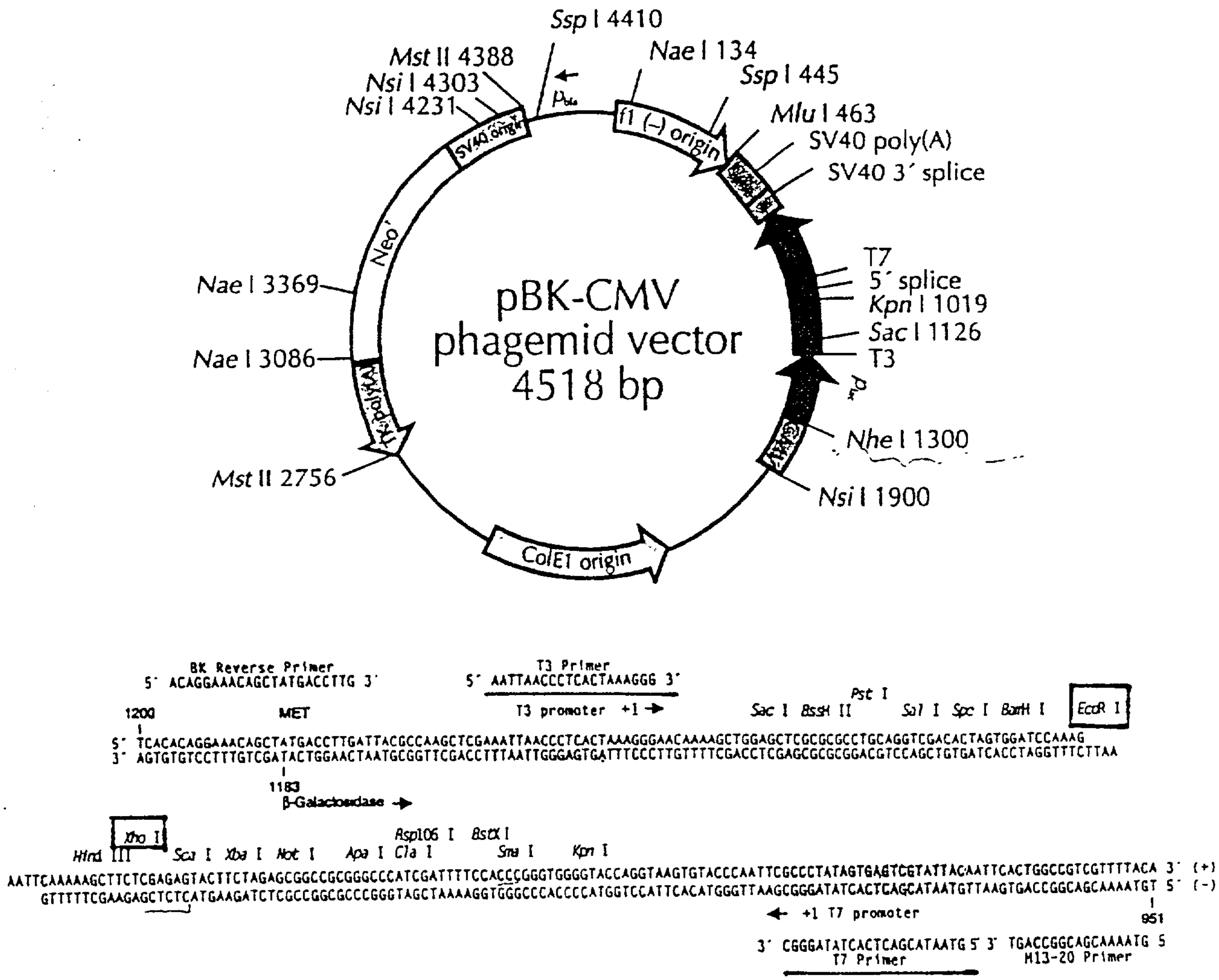

Ssp I 4410
Nae I 134
Ssp I 445
Mlu I 463
SV40 poly(A)
SV40 3´ splice
Mst II 4388
Nsi I 4303
Nsi I 4231
SV40 origin
f1 (-) origin
Neo
T7
5´ splice
Kpn I 1019
Sac I 1126
T3
Nae I 3369
Nae I 3086
pBK-CMV
phagemid vector
4518 bp
TK poly(A)
Nhe I 1300
Mst II 2756
Nsi I 1900
ColE1 origin
BK Reverse Primer
5´ ACAGGAAACAGCTATGACCTTG 3´
T3 Primer
5´ AATTAACCCTCACTAAAGGG 3´
T3 promoter +1
1200
MET
Sac I BssH II Pst I Sal I Spe I BamH I EcoR I
5´ TCACACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCGCGCGCCTGCAGGTCGACACTAGTGGATCCAAAG
3´ AGTGTGTCCTTTGTCGATACTGGAACTAATGCGGTTCGACCTTTAATTGGGAGTGATTTCCCTTGTTTTCGACCTCGAGCGCGCGGACGTCCAGCTGTGATCACCTAGGTTTCTTAA
1183
β-Galactosidase
Xho I
Hind III Sca I Xba I Not I Apa I Cla I Asp106 I BstX I Sma I Kpn I
AATTCAAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCGCGGGCCCATCGATTTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACA 3´ (+)
GTTTTTCGAAGAGCTCTCATGAAGATCTCGCCGGCGCCCGGGTAGCTAAAAGGTGGGCCCACCCCATGGTCCATTCACATGGGTTAAGCGGGATATCACTCAGCATAATGTTAAGTGACCGGCAGCAAAATGT 5´ (-)
+1 T7 promoter
951
3´ CGGGATATCACTCAGCATAATG 5´
T7 Primer
3´ TGACCGGCAGCAAAATG 5´
M13-20 Primer

FIG. 2.4 b

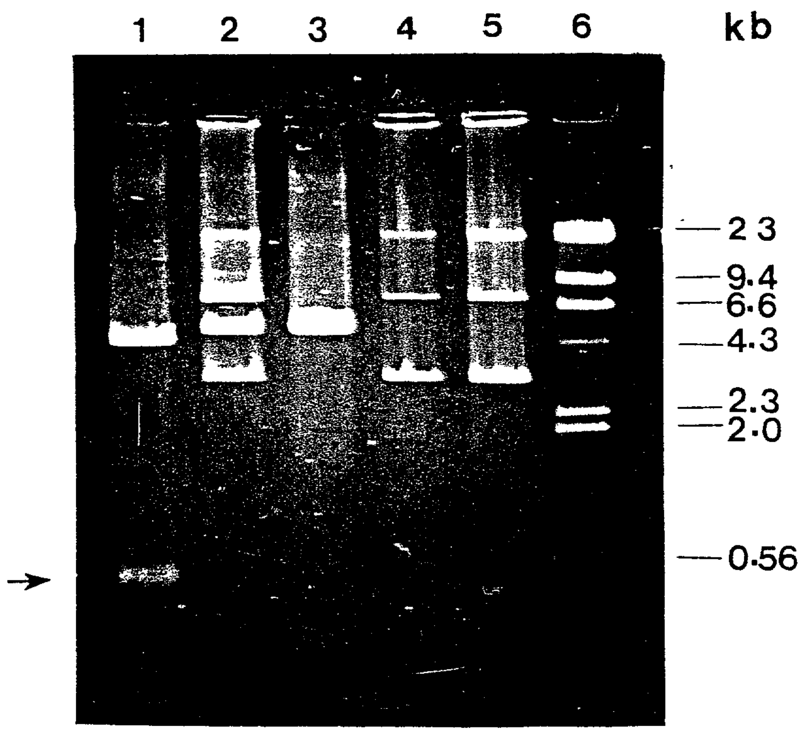


FIG 2.5

DNA sequence of Tm 13.17 cDNA clone

```
          B         E
          a         c
          m         o
          H         R
          I         I
  1 AGTGGATCCAAAGAATTCGGCACGAGACTACTAAGATGAAGTTGCTCTGTTGTCTAATCT
                                       M  K  L  L  C  C  L  I  S

 61 CCCTCATTCTGTTGGTCACAGTTCAGGCCCTGACCGAGGCACAAATTGAGAAACTGAACA
      L  I  L  L  V  T  V  Q  A  L  T  E  A  Q  I  E  K  L  N  K

121 AGATCAGCAAAAAATGTCAAAATGAAAGTGGAGTGTCGCAAGAGATCATAACCAAAGCTC
      I  S  K  K  C  Q  N  E  S  G  V  S  Q  E  I  I  T  K  A  R

181 GCAACGGTGACTGGGAGGACGATCCTAAACTGAAACGCCAAGTTTTTTGCGTGGCCAGGA
      N  G  D  W  E  D  D  P  K  L  K  R  Q  V  F  C  V  A  R  N

241 ACGCCGGTCTGGCCACGGAATCGGGAGAGGTGGTGGTCGACGTGTTGAGGGAGAAGGTGA
      A  G  L  A  T  E  S  G  E  V  V  V  D  V  L  R  E  K  V  R

301 GGAAGGTCACTGACAACGACGAAGAAACTGAGAAAATCATCAATAAGTGCGCCGTCAAGA
      K  V  T  D  N  D  E  E  T  E  K  I  I  N  K  C  A  V  K  R

361 GAGATACTGTTGAAGAGACGGTGTTCAATACTTTCAAATGTGTCATGAAAAACAAGCCAA
      D  T  V  E  E  T  V  F  N  T  F  K  C  V  M  K  N  K  P  K

421 AGTTCTCACCAGTTGATTGAACCACCACGACTAGTAGATGGTTCAAATGGTGTGCTTTAC
      F  S  P  V  D  *

                                                              X
                                                              h
                                                              o
                                                              I
481 ATATAAAAATAAAGTGTTTCTGATGTAAAAAAAAAAAAAAAAAAAAAAAAAACTCG
         polyadenylation signal  poly (A) tail (26)

537 AGAGTATTCTAGAGCGGCCGCGGGCCCATCGTTTTCCACCC
```

FIG 2.6a

$$\underbrace{\text{M K}}_{\substack{\text{n-region}\\ \text{(basic)}}}\ \underbrace{\text{L L C C L I S L I C C V}}_{\substack{\text{h-region}\\ \text{(central hydrophobic)}}}\ \underbrace{\text{T V Q A}}_{\substack{\text{c-region}\\ \text{(more polar)}}}\overset{\text{cleavage site}}{\downarrow}$$

FIG 2.6b

A. Mature Tm 13.17 amino acid residure

```
  1  LTEAQIEKLN KISKKCQNES GVSQEIITKA RNGDWEDDPK LKRQVFCVAR
 51  NAGLATESGE VVVDVLREKV RKVTDNDEET EKIINKCAVK RDTVEETVFN
101  TFKCVMKNKP KFSPVD
```

B. Summary of the composition analysis for the mature Tm 13.17 sequence:

Residue	Number	Mole Percent
A = Ala	6	5.172
B = Asx	0	0.000
C = Cys	4	3.448
D = Asp	8	6.897
E = **Glu**	**13**	**11.207**
F = Phe	4	3.448
G = Gly	4	3.448
H = His	0	0.000
I = Ile	6	5.172
K =**Lys**	**16**	**13.793**
L = Leu	5	4.310
M = Met	1	0.862
N = Asn	8	6.897
P = Pro	3	2.586
Q = Gln	4	3.448
R = Arg	6	5.172
S = Ser	5	4.310
T = Thr	8	6.897
V **=Val**	**14**	**12.069**
W = Trp	1	0.862
Y = Tyr	0	0.000
Z = Glx	0	0.000

Molecular weight = 13171.96; Residues = 116; Average Residue Weight = 113.551

Charge = 1; Isoelectric point = 7.74.

FIG 2.6c

```
  1 AGTGGATCCAAAGAATTCGGCACGAGACTACTAAGATGAA   Tm 13.17
                                                B 1

 41 GTTGCTCTGTTGTCTAATCTCCCTCATTCTGTTGGTCACA   Tm 13.17
  1              CTTACTTCTCTCATTCTGTTGGTCCCA   B 1

 81 GTTCAGGCCCTGACCGAGGCACAAATTGAGAAACTGAACA   Tm 13.17
 28 GTTCAGGCCATAACTCAGGAAGACCTTGAGCTACTGCCCC   B 1

121 AGATCAGCAAAAAATGTCAAAATGAAAGTGGAGTGTCGCA   Tm 13.17
 68 AAACCAGCGCAGAGTGCAAGACTGAAAGTGGAGTGTCCGA   B 1

161 AGAGATCATAACCAAAGCTCGCAACGGTGACTGGGAGGAC   Tm 13.17
118 AGACGTCATAAAGAGAGCTCGCAAAGGTGACTTGGAGGAC   B 1

201 GATCCTAAACTGAAACGCCAAGTTTTTTGCGTGGCCAGGA   Tm 13.17
148 GACCCCAAACTGAAAATGCAACTTCTTTGCATTTTCAAGG   B 1

241 ACGCCGGTCTGGCCACGGAATCGGGAGAGGTGGTGGTCGA   Tm 13.17
188 CACTCGAAATAGTCGCGGAATCGGGAGAAATTGAGGCCGA   B 1

281 CGTGTTGAGGGAGAAGGTGAGGAAGGTCACTGACAACGAC   Tm 13.17
228 CACGTTCAAGGAGAAGTTGACGA GGTGACAAACGATGAT   B 1

321 GAAGAAACTGAGAAAATCATCAATAAGTGCGCCGTCAAGA   Tm 13.17
268 GAAGAAAGCGAGAAGATTGTCGAGAAGTGCACGGTGACTG   B 1

361 GAGATACTGTTGAAGAGACGGTGTTCAATACTTTCAAATG   Tm 13.17
308 AACACACTCCGGAAGATACGCCATTTGAAGTTACCAAATG   B 1

                                      *
401 TGTCATGAAAAACAAGCCAAAGTTCTCACCAGTTGATTGA   Tm 13.17
348 TGTATTGAAGGACAAGCCCAATTTCT          TTGC   B 1

441 ACCACCACGACTAGTAGATGGTTCAAATGGTGTGCTTTAC   Tm 13.17
388 A       GAC   TATTTGTCTGAAA      GCTTTGT   B 1

481 ATATAAAAATAAAGTGTTTCTGATGTAAAAAAAAAAAAAA   Tm 13.17
428 GCACAA                                     B 1
```

FIG 2.7

```
Tm 13.17     3  EAQIEKLNKISKKCQNESGVSQEIITKARNGDWEDDPKLKRQVFCVARNA 52
                |.. |||.. |. |..||||||:| :.|.|| :  |||||| :.||: :.|
AFP-3        1  ETPREKLKQHSDACKAESGVSEESLNKVRNREEVDDPKLKEHAFCILKRA 50

Tm 13.17    53  GLATESGEVVVDVLREKVRKVTDNDEETEKIINKCAVKRDTVEETVFNTF 102
                |: ..|||. :| ::.|.:. .::.|..:.::.|||||:||.:..  : |
AFP-3       51  GFIDASGEFQLDHIKTKFKENSEHPEKVDDLVAKCAVKKDTPQHSSADFF 100

Tm 13.17   103  KCVMKNKP 110
                ||| .|:.
AFP-3      101  KCVHDNRS 108
```

Percent identity: 39.8 (identical amino acids; Percent similarity: 58.3 (identical amino acids plus conservative amino acids).

FIG 2.8

Tm 13.17	M K L L C C L I S L I L L V T V Q A
AFP-3	M K L L L C L V L V A L V A A T Y A
B Protein	L T S L I L L V A V Q A

FIG 2.9

```
Tm 13.17   NH2-L T E A Q I E K L N K I S K K C Q N E
                   :   .     :                 .
Tm 12.86   NH2-L T D E Q I Q K R N K I S K E ? Q Q V
```

FIG 2.10

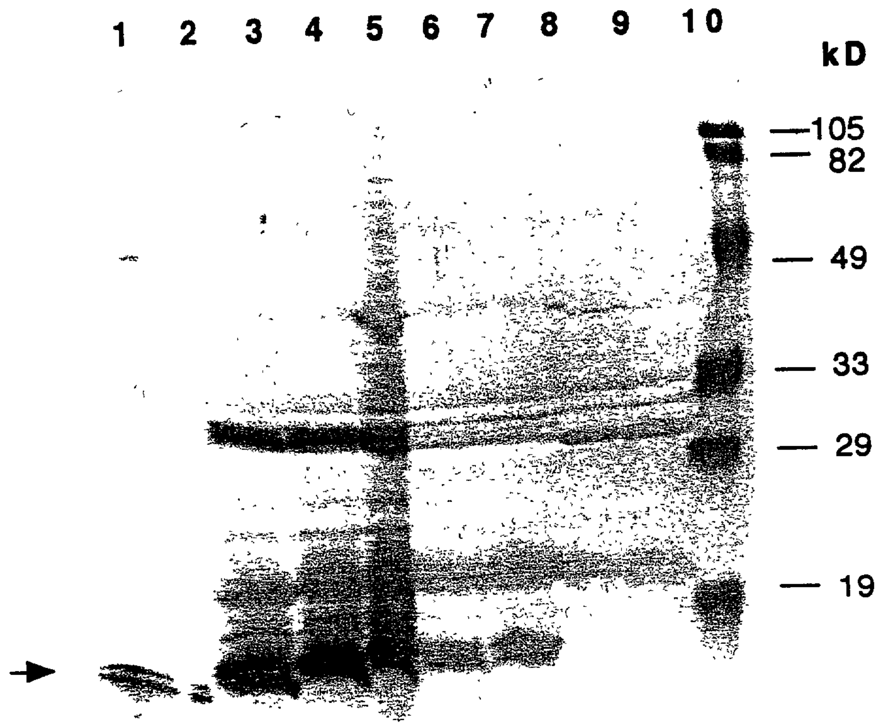


FIG 2.11

```
Tm 12.86       L T D E Q I Q K R N K I S K E ? Q Q V
Tm 13.17   1   L T E A Q I E K L N K I S K K C Q N E S G V S Q E I I T K A    30
B1        13   I T E E D L E L L R Q T S A E C K T E S G V S E D V I K R A    43
AFP-3      1       E T P R E K L K Q H S D A C K A E S G V S E E S L N K V    28

Tm13.17   31   R N G D W E D D P K L K R Q V F C V A R N A G L A T E S G E    60
B1        44   R K G D L E D D P K L K M Q L L C I F K A L E I V A E S G E    74
AFP-3     29   R N R E E V D D P K L K E H A F C I L K R A G F I D A S G E    58

Tm13.17   61   V V V D V L R E K V R K V T D N D E E T E K I I N K C A V K    90
B1        75   I E A D T F K E K L T R V T N D D E E S E K I V E K C T V T   105
AFP-3     59   F Q L D H I K T K F K E N S E H P E K V D D L V A K C A V K    88

Tm13.17   91   R D T V E E T V F N T F K C V M K N K P K F S P V D           116
B1       106   E D T P E D T A F E V T K C V L K D K P N F F G D L F V       124
AFP-3     89   K D T P Q H S S A D F F K C V H D N R S                       108
```

FIG 2.12

```
1    GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCGTTCGCCGCC
                  M  K  L  L  C  F  A  F  A  A

47   ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA
     I  V  I  G  A  Q  A  L  T  D  E  Q  I  Q  K

92   AGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGTGTCC
     R  N  K  I  S  K  E  C  Q  Q  V  S  G  V  S

137  CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGATGAT
     Q  E  T  I  D  K  V  R  T  G  V  L  V  D  D

182  CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGA
     P  K  M  K  K  H  V  L  C  F  S  K  K  T  G

226  GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
     V  A  T  E  A  G  D  T  N  V  E  V  L  K  A

271  AAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTG
     K  L  K  H  V  A  S  D  E  E  V  D  K  I  V

316  CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
     Q  K  C  V  V  K  K  A  T  P  E  E  T  A  Y

361  GACACCTTCAAGTGTATTTACGACAGCAAACCTGATTTCTCTCCT
     D  T  F  K  C  I  Y  D  S  K  P  D  F  S  P

406  ATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT
     I  D  *
                                    polyadenylation signal

451  ACTATCGTTATGTAAAAAAAAAAAAAAAAAA
```

poly (A) tail

FIG 3.0

```
1    GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCTTTCGCCGCC
                  M  K  L  L  L  C  F  A  F  A  A

47   ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA
     I  V  I  G  A  Q  A  L  T  D  E  Q  I  Q  K

92   AGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGTGTCC
     R  N  K  I  S  K  E  C  Q  Q  V  S  G  V  S

137  CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT
     Q  E  T  I  D  K  V  R  T  G  V  L  V  D  D

182  CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGA
     P  K  M  K  K  H  V  L  C  F  S  K  K  T  G

226  GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
     V  A  T  E  A  G  D  T  N  V  E  V  L  K  A

271  AAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTG
     K  L  K  H  V  A  S  D  E  E  V  D  K  I  V

316  CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
     Q  K  C  V  V  K  K  A  T  P  E  E  T  A  Y

361  GACACCTTCAAGTGTATTTACGACAGTAAACCTGATTTCTCTCCT
     D  T  F  K  C  I  Y  D  S  K  P  D  F  S  P

406  ATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT
     I  D  *
                                    polyadenylation signal

451  ACTATCGTTATGAAAAAAAAAAAAAAAAAAA
```

poly (A) tail

FIG 3.1

```
                                start
                                  ↓
2-2   G G C A C G A G C A A A A A T G A A A C T C C T C T T G T G C T T T G C G
2-3   G G C A C G A G C A A A A A T G A A A C T C C T C T T G T G C T T T G C T

2-2   T T C G C C G C C A T C G T C A T C G G A G C T C A G G C T C T C A C C G
2-3   T T C G C C G C C A T C G T C A T C G G A G C T C A G G C T C T C A C C G

2-2   A C G A A C A G A T A C A G A A A A G G A A C A A G A T C A G C A A A G A
2-3   A C G A A C A G A T A C A G A A A A G G A A C A A G A T C A G C A A A G A

2-2   A T G C C A G C A G G T G T C C G G A G T G T C C C A A G A G A C G A T C
2-3   A T G C C A G C A G G T G T C C G G A G T G T C C C A A G A G A C G A T C

2-2   G A C A A A G T C C G C A C A G G T G T C T T G G T C G A T G A T C C C A
2-3   G A C A A A G T C C G C A C A G G T G T C T T G G T C G A C G A T C C C A

2-2   A A A T G A A G A A G C A C G T C C T C T G C T T C T C G A A G A A A A C
2-3   A A A T G A A G A A G C A C G T C C T C T G C T T C T C G A A G A A A A C

2-2   T G G A G T G G C A A C C G A A G C C G G A G A C A C C A A T G T G G A G
2-3   T G G A G T G G C A A C C G A A G C C G G A G A C A C C A A T G T G G A G

2-2   G T A C T C A A A G C C A A G C T G A A G C A T G T G G C C A G C G A C G
2-3   G T A C T C A A A G C C A A G C T G A A G C A T G T G G C C A G C G A C G

2-2   A A G A G G T G G A C A A G A T C G T G C A G A A G T G C G T G G T C A A
2-3   A A G A A G T G G A C A A G A T C G T G C A G A A G T G C G T G G T C A A

2-2   G A A G G C C A C A C C A G A G G A A A C G G C T T A T G A C A C C T T C
2-3   G A A G G C C A C A C C A G A G G A A A C G G C T T A T G A C A C C T T C

2-2   A A G T G T A T T T A C G A C A G C A A A C C T G A T T T C T C T C C T A
2-3   A A G T G T A T T T A C G A C A G T A A A C C T G A T T T C T C T C C T A

                *
2-2   T T G A T T A A T T G T T T T G T A T T T G A C T G A A T T T T G A C A A
2-3   T T G A T T A A T T G T T T T G T A T T T G A C T G A A T T T T G A C A A

2-2   T A A A G G T A A T A T C G T T A T G T A A A A A
2-3   T A A A G G T A C T A T C G T T A T G A A A A A A
```

FIG 3.2

Predicted Amino Acid

Composition of 2-2 and 2-3

Analysis	Whole Protein
Molecular Weight	12843.80 m.w.
Length	115
1 microgram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	48	47.19	41.74
Acidic (DE)	20	18.90	17.39
Basic (KR)	20	20.40	17.39
Polar (NCQSTY)	30	25.35	26.09
Hydrophobic (AILFWV)	34	27.26	29.57
A Ala	6	3.32	5.22
C Cys	4	3.21	3.48
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
I Ile	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.98	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.08	7.83
V Val	14	10.80	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

FIG 3.3

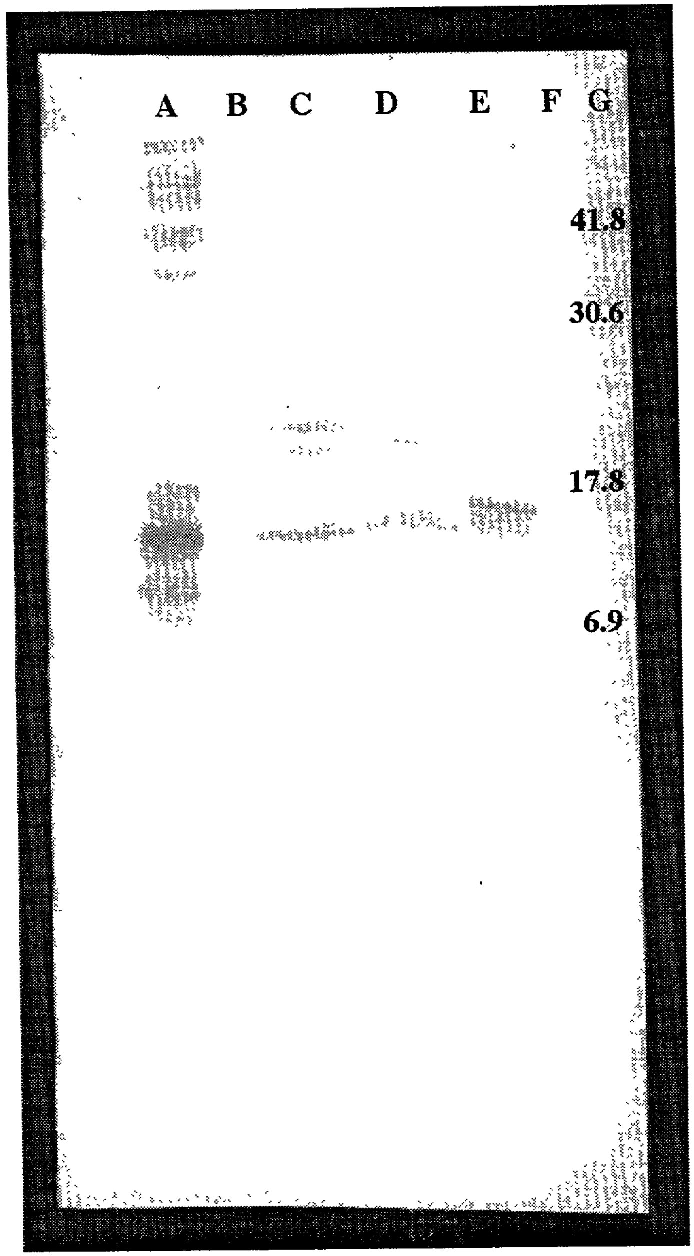


FIG 3.4

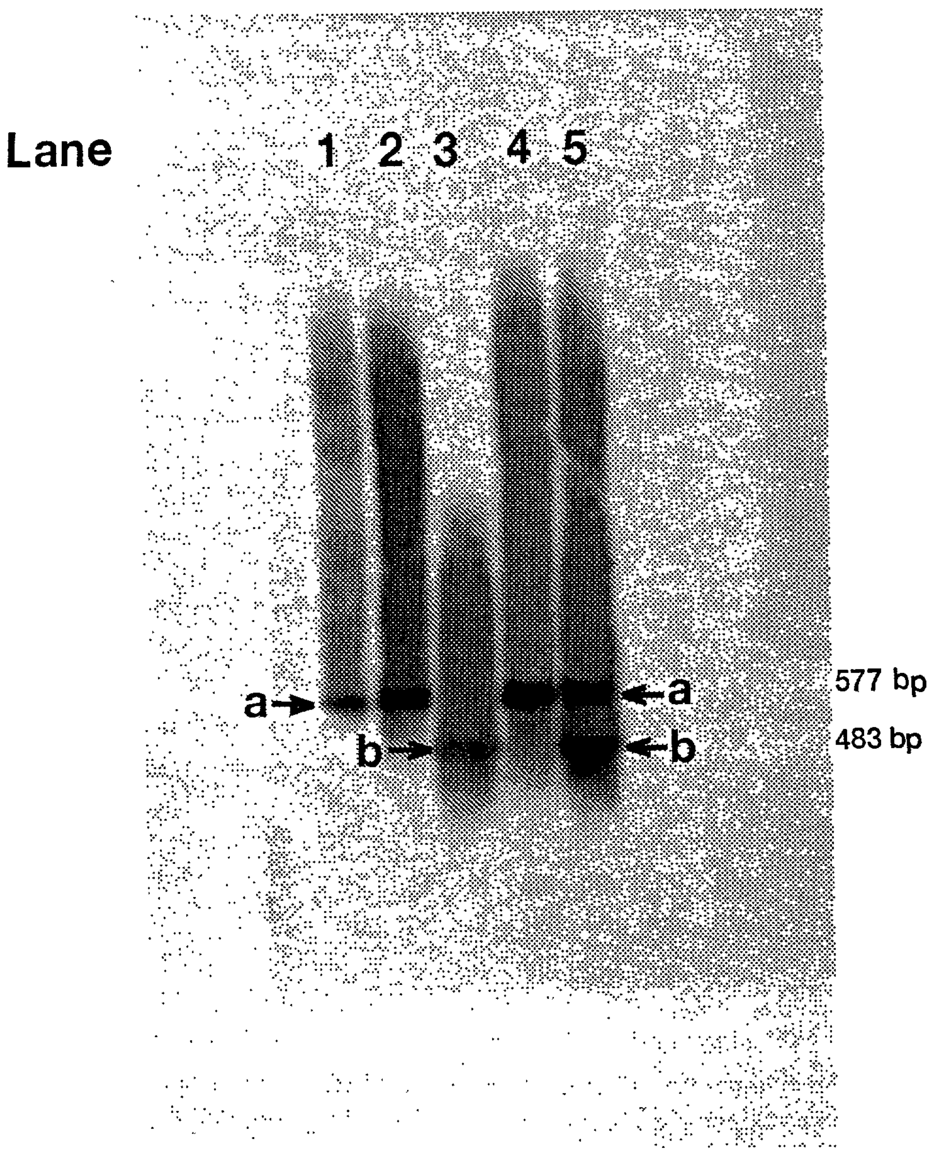


FIG 4.0

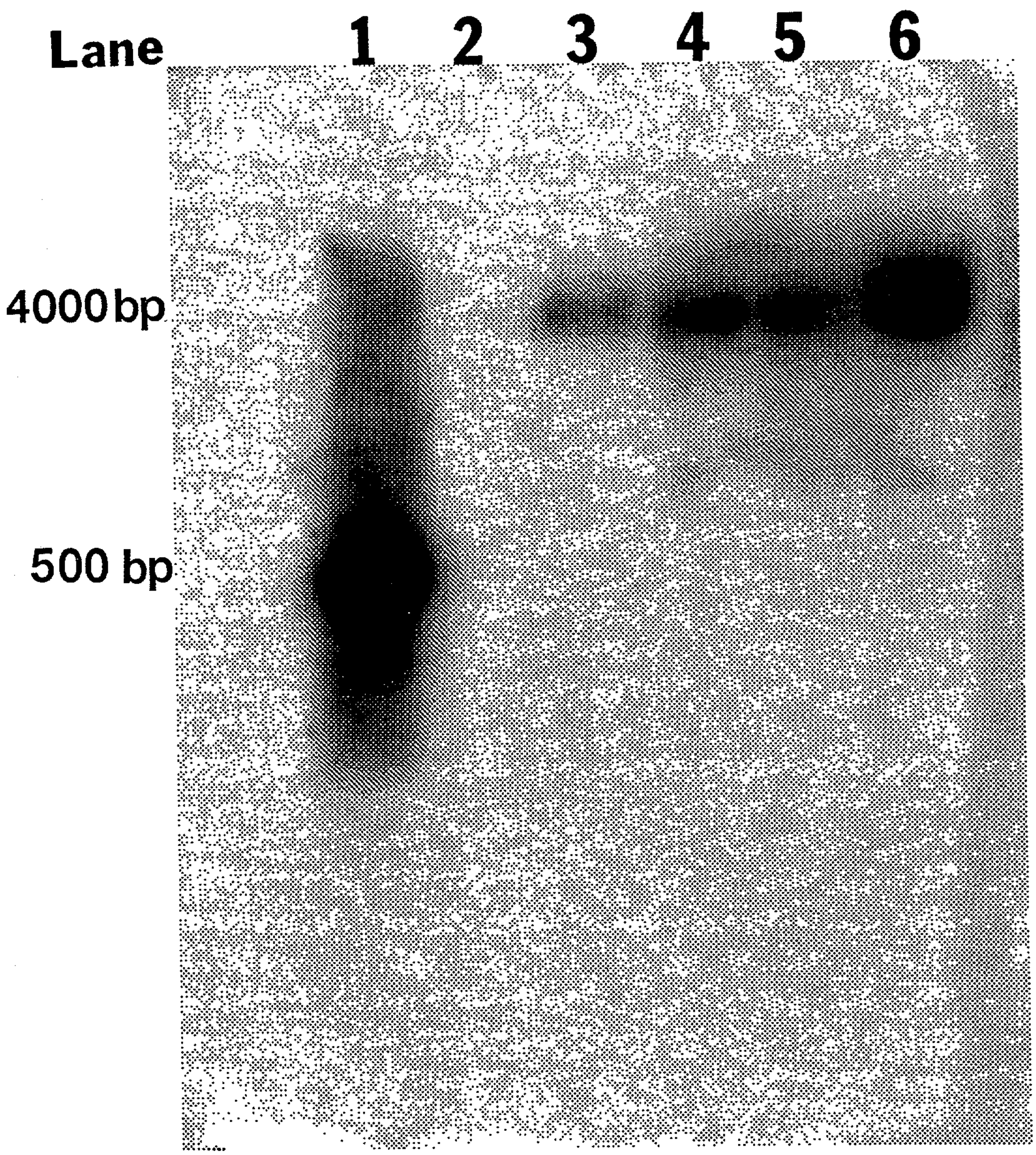


FIG 4.1

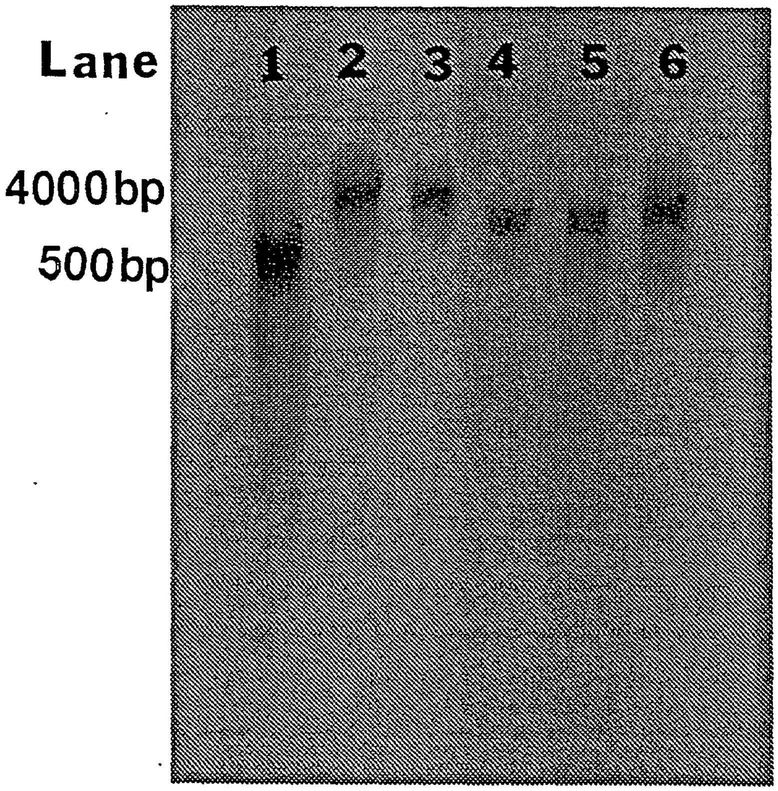


FIG 4.2

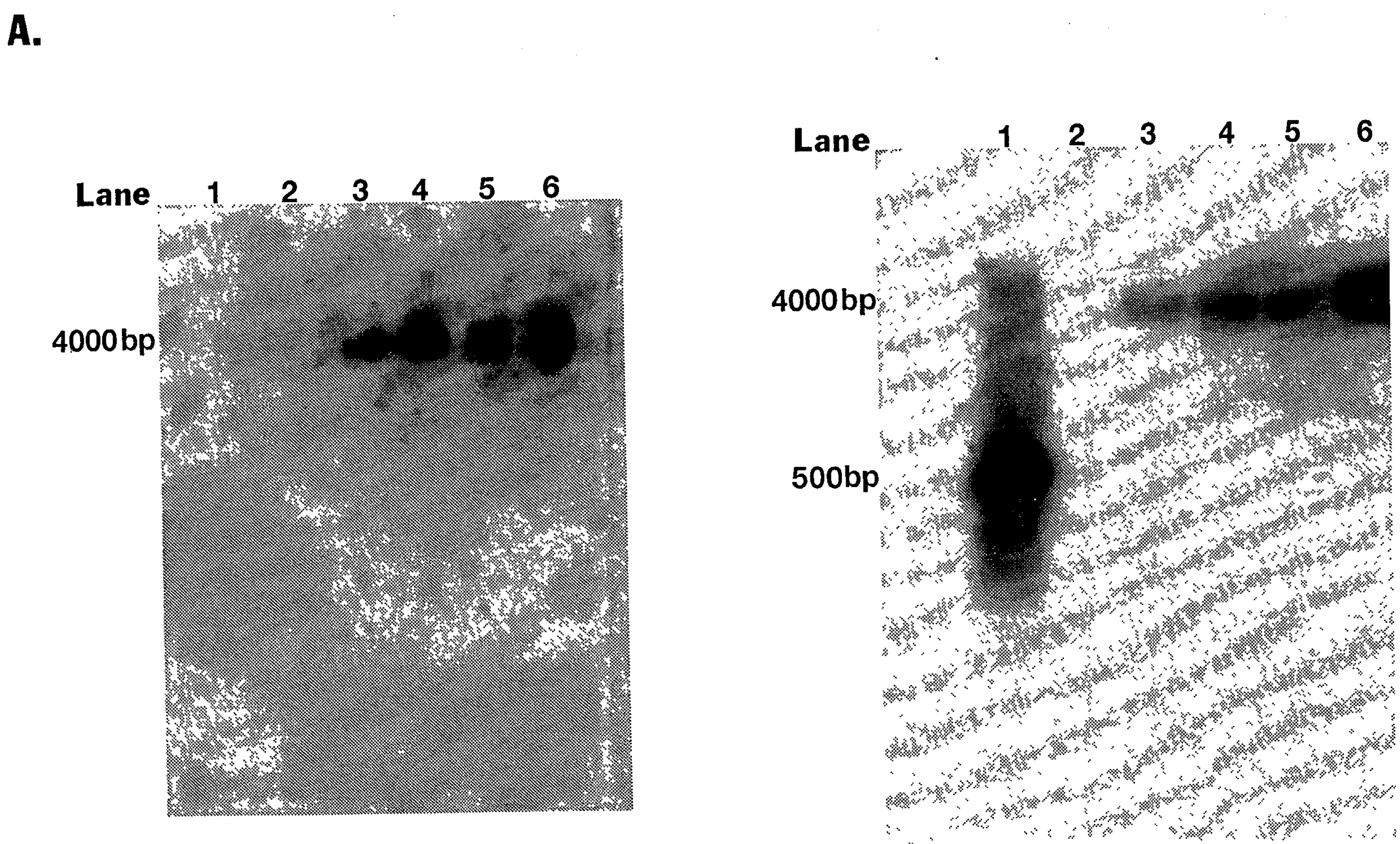


FIG 4.3

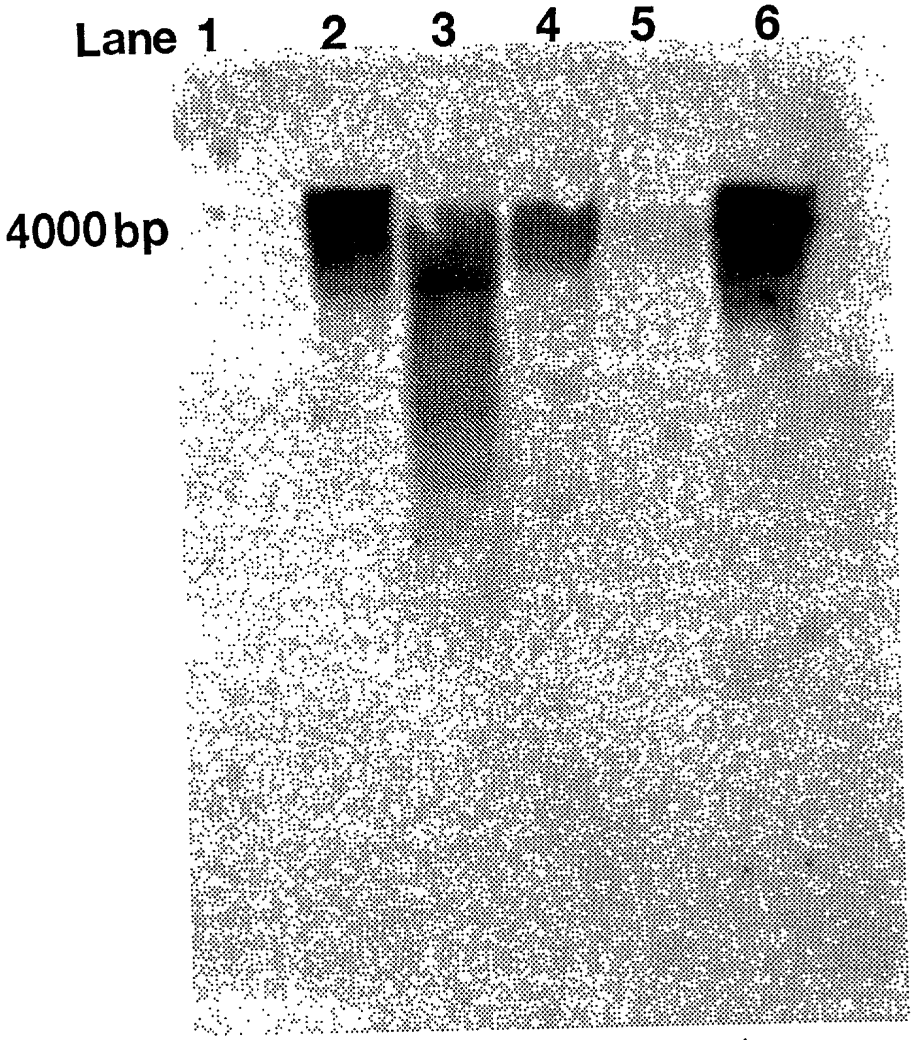


FIG 4.4

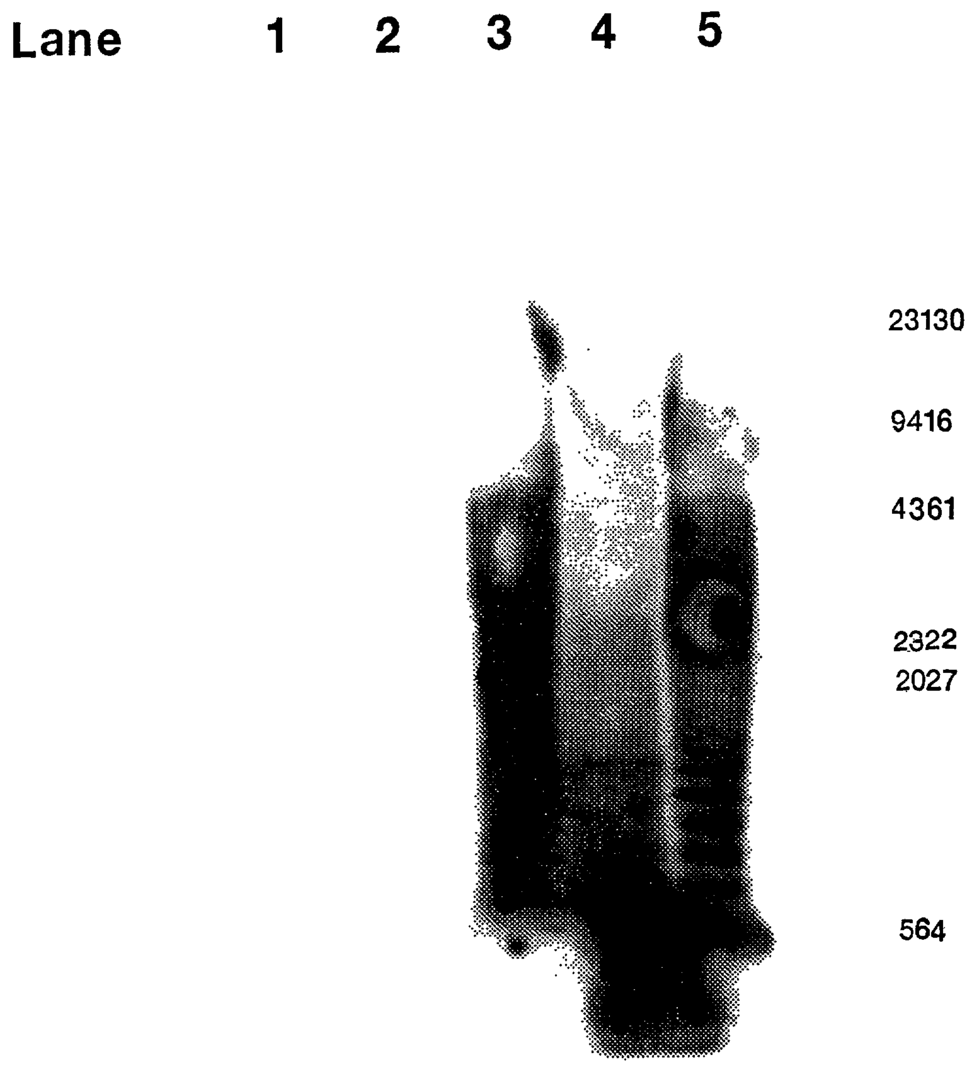


FIG 4.5

Tm 13.17 cDNA

```
  1 AGTGGATCCAAAGAATTCGGCACGAGACTACTAAGATGAAGTTGCTCTGTTGTCTAATCT
                                        M  K  L  L  C  C  L  I  S

 61 CCCTCATTCTGTTGGTCACAGTTCAGGCCCTGACCGAGGCACAAATTGAGAAACTGAACA
     L  I  L  L  V  T  V  Q  A  L  T  E  A  Q  I  E  K  L  N  K
                                 Forward Primer

121 AGATCAGCAAAAAATGTCAAAATGAAAGTGGAGTGTCGCAAGAGATCATAACCAAAGCTC
     I  S  K  K  C  Q  N  E  S  G  V  S  Q  E  I  I  T  K  A  R

181 GCAACGGTGACTGGGAGGACGATCCTAAACTGAAACGCCAAGTTTTTTGCGTGGCCAGGA
     N  G  D  W  E  D  D  P  K  L  K  R  Q  V  F  C  V  A  R  N

241 ACGCCGGTCTGGCCACGGAATCGGGAGAGGTGGTGGTCGACGTGTTGAGGGAGAAGGTGA
     A  G  L  A  T  E  S  G  E  V  V  V  D  V  L  R  E  K  V  R

301 GGAAGGTCACTGACAACGACGAAGAAACTGAGAAAATCATCAATAAGTGCGCCGTCAAGA
     K  V  T  D  N  D  E  E  T  E  K  I  I  N  K  C  A  V  K  R
     Reverse Primer
361 GAGATACTGTTGAAGAGACGGTGTTCAATACTTTCAAATGTGTCATGAAAAACAAGCCAA
     D  T  V  E  E  T  V  F  N  T  F  K  C  V  M  K  N  K  P  K

421 AGTTCTCACCAGTTGATTGAACCACCACGACTAGTAGATGGTTCAAATGGTGTGCTTTAC
     F  S  P  V  D  *

481 ATATAAAAATAAAGTGTTTCTGATGTAAAAAAAAAAAAAAAAAAAAAAAAAACTC
```

FIG. 4.6 a

B.

Forward Primer

2-2	L T D E Q I Q K R N K I S K E C Q Q V S G V S Q E T I D K V R T G V L V
Tm 13.17	L T E A Q I E K L N K I S K K C Q N E S G V S Q E I I T K A R N G D W E
B2	L T E E D L Q L L R Q T S A E C K T E S G A S E A V I K K A R K G D L E
AFP-3	E T P R E K L K Q H S D A C K A E S G V S E E S L N K V R N R E E V

2-2	D D P K M K K H V L C F S K K T G V A T E A G D T N V E V L K A K L K H
Tm 13.17	D D P K L K R Q V F C V A R N A G L A T E S G E V V V D V L R E K V R K
B2	D D P K L K M Q L L C I F K A L E I V A E S G E I E A D T F K E K L T R
AFP-3	D D P K L K E H A F C I L K R A G F I D A S G E F Q L D H I K T K F K E

Reverse Primer

2-2	V A S D E E V D K I V Q K C V V K K A T P E E T A Y D T F K C I Y D S
Tm 13.17	V T D N D E E T E K I I N K C A V K R D T V E E T V F N T F K C V M K N
B2	V T N D D E E S E K I V E K C T V T E D T P E D T A F E V T K C V L K D
AFP-3	N S E H P E K V D D L V A K C A V K K D T P Q H S S A D F F K C V H D N

2-2	K P D F S P I D
Tm 13.17	K P K F S P V D
B2	K P N F F G D L F V
AFP-3	R S

C.

Primer	percent % composition A	C	G	T	Melting Temperature (°C)
Forward	28.6	14.3	42.9	14.3	44.0
Reverse	25.0	31.3	6.3	37.5	44.0

FIG 4.6

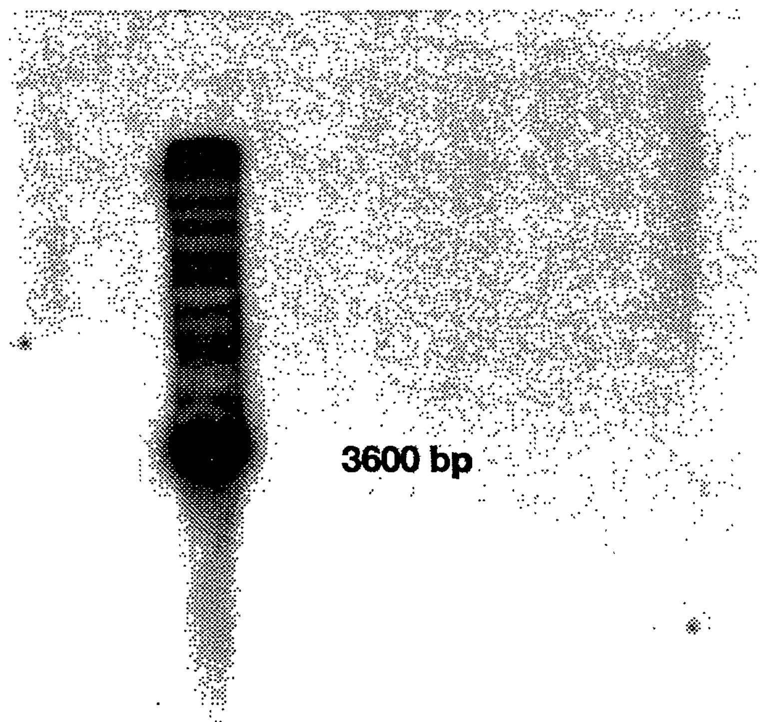


FIG 4.7

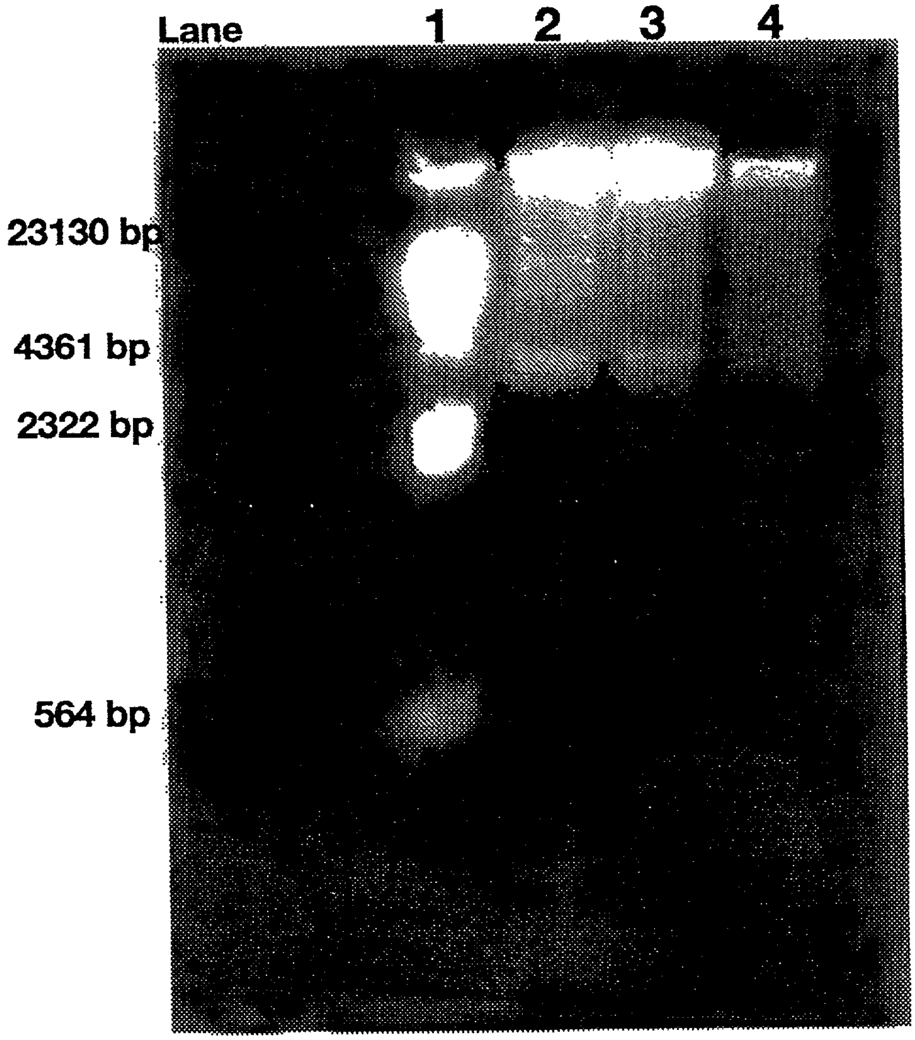


FIG 4.8

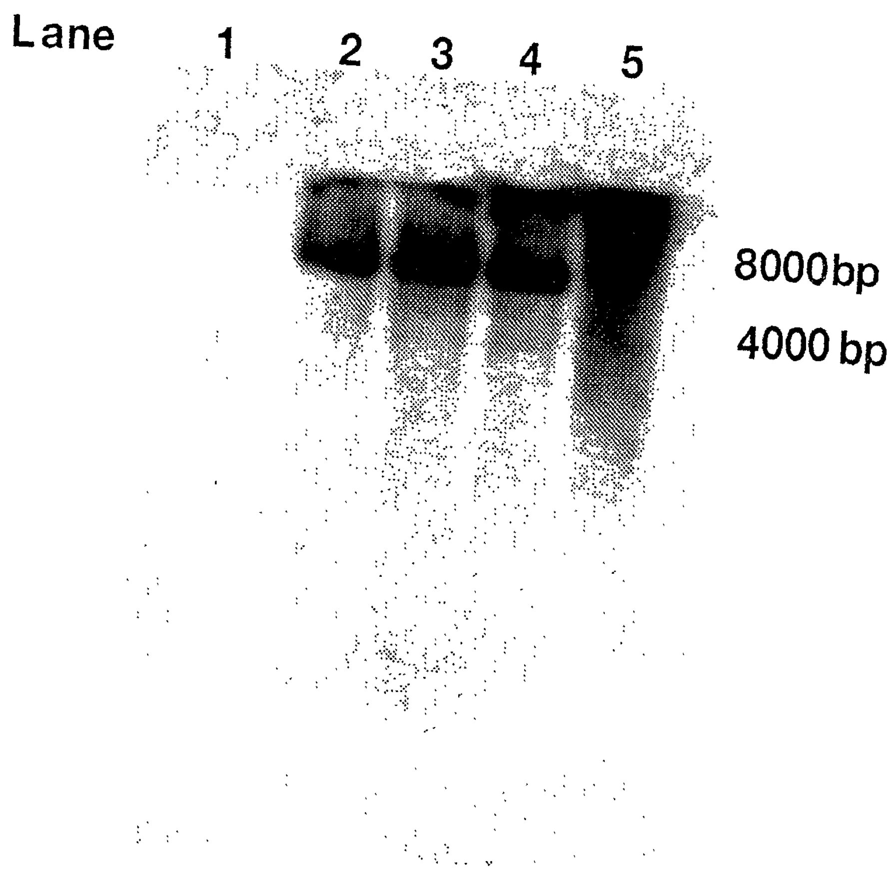


FIG 4.9

```
1    GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCTTTCGCCGCC
                  M  K  L  L  C  F  A  F  A  A

47   ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA
     I  V  I  G  A  Q  A  L  T  D  E  Q  I  Q  K

92   AGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGTGTCC
     R  N  K  I  S  K  E  C  Q  Q  V  S  G  V  S

137  CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT
     Q  E  T  I  D  K  V  R  T  G  V  L  V  D  D

182  CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGA
     P  K  M  K  K  H  V  L  C  F  S  K  K  T  G

226  GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
     V  A  T  E  A  G  D  T  N  V  E  V  L  K  A

271  AAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTG
     K  L  K  H  V  A  S  D  E  E  V  D  K  I  V

316  CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
     Q  K  C  V  V  K  K  A  T  P  E  E  T  A  Y

361  GACACCTTCAAGGTTATTTACGACAGTAAACCTGATTTCTCTCCT
     D  T  F  K  V  I  Y  D  S  K  P  D  F  S  P

406  ATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT
     I  D  *
                                   polyadenylation signal
451  ACTATCGTTATGTAAAAAAAAAAAAAAAAA

                    poly (A) tail
```

FIG. 4.10 a

Predicted Amino Acid Composition of 3-4

Analysis	Whole Protein
Molecular Weight	12839.70 m.w.
Length	115
1 microgram =	77.883 pMoles
Molar Extinction coefficient	2920±5%
1 A(280) =	4.40 mg/ml
Isoelectric Point	7.14
Charge at pH 7	0.16

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	47	46.41	40.87
Acidic (DE)	20	18.91	17.39
Basic (KR)	20	20.41	17.39
Polar (NCQSTY)	29	24.55	25.22
Hydrophobic (AILFWV)	35	28.04	30.43
A Ala	6	3.32	5.22
C Cys	3	2.41	2.61
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
I Ile	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.99	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.09	7.83
V Val	15	11.58	13.04
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

FIG. 4.10 b

```
1    GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCTTTCGCCGCC
                  M  K  L  L  L  C  F  A  F  A  A

47   ATCGTCATCGGAGCTCAGGCTCTCACCGATGAACAGATACAGAAA
     I  V  I  G  A  Q  A  L  T  D  E  Q  I  Q  K

92   AGGAACAAGATCAGCAAAGAATGCCAGCAGGAGTCCGGAGTGTCC
     R  N  K  I  S  K  E  C  Q  Q  E  S  G  V  S

137  CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT
     Q  E  T  I  D  K  V  R  T  G  V  L  V  D  D

182  CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAGAACTGGA
     P  K  M  K  K  H  V  L  C  F  S  K  R  T  G

226  GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
     V  A  T  E  A  G  D  T  N  V  E  V  L  K  A

271  AAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTG
     K  L  K  H  V  A  S  D  E  E  V  D  K  I  V

316  CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
     Q  K  C  V  V  K  K  A  T  P  E  E  T  A  Y

361  GACACCTTCAAGTGTATTTACGACAGTAAACCTGATTTCTCTCCT
     D  T  F  K  V  I  Y  D  S  K  P  D  F  S  P

406  ATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT
     I  D  *
                                   polyadenylation signal

451  ACTATCGTTATGAAAAAAAAAAAAAAAAAA
```

poly (A) tail

FIG. 4.11 a

Predicted Amino Acid

Composition of 3-9

Analysis	Whole Protein
Molecular Weight	12871.80 m.w.
Length	115
1 microgram =	77.689 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.23 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	48	47.31	41.74
Acidic (DE)	20	18.86	17.39
Basic (KR)	20	20.57	17.39
Polar (NCQSTY)	30	25.29	26.09
Hydrophobic (AILFWV)	34	27.20	29.57
A Ala	6	3.31	5.22
C Cys	4	3.21	3.48
D Asp	11	9.84	9.57
E Glu	9	9.03	7.83
F Phe	3	3.43	2.61
G Gly	4	1.77	3.48
H His	2	2.13	1.74
I Ile	6	5.28	5.22
K Lys	17	16.93	14.78
L Leu	5	4.40	4.35
M Met	1	1.02	0.87
N Asn	2	1.77	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.97	5.22
R Arg	3	3.64	2.61
S Ser	7	4.74	6.09
T Thr	9	7.07	7.83
V Val	14	10.78	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

FIG. 4.11 b

```
1    GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCGTTCGCCGCC
                  M  K  L  L  L  C  F  A  F  A  A

47   ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA
     I  V  I  G  A  Q  A  L  T  D  E  Q  I  Q  K

92   AGGAACAAGATCAGCAAAGAGTGCCAGCAGGTGTCCGGAGTGTCC
     R  N  K  I  S  K  E  C  Q  Q  E  S  G  V  S

137  CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT
     Q  E  T  I  D  K  V  R  T  G  V  L  V  D  D

182  CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGA
     P  K  M  K  K  H  V  L  C  F  S  K  R  T  G

226  GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
     V  A  T  E  A  G  D  T  N  V  E  V  L  K  A

271  AAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTG
     K  L  K  H  V  A  S  D  E  E  V  D  K  I  V

316  CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
     Q  K  C  V  V  K  K  A  T  P  E  E  T  A  Y

361  GACACCTTCAAGTGTATTTACGACAGTAAACCTGATTTCTCTCCT
     D  T  F  K  V  I  Y  D  S  K  P  D  F  S  P

406  ATTGATTAATTGTTTTGTATTTGGCTGAATTTTGACAATAAAGGT
     I  D  *
                                        polyadenylation signal

451  ACTATCGTTATGTAAAAAAAAAAAAAAAAAA
```

poly (A) tail

FIG. 4.12 a

Predicted Amino Acid

Composition of 7–5

Analysis	Whole Protein
Molecular Weight	12843.80 m.w.
Length	115
1 microgram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	48	47.19	41.74
Acidic (DE)	20	18.90	17.39
Basic (KR)	20	20.40	17.39
Polar (NCQSTY)	30	25.35	26.09
Hydrophobic (AILFWV)	34	27.26	29.57
A Ala	6	3.32	5.22
C Cys	4	3.21	3.48
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
I Ile	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.98	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.08	7.83
V Val	14	10.80	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

FIG. 4.12 b

```
                          ↓
2-2  GGCACGAGCAAAAAATGAAACTCCTCTTGTGCTTTGCGTTCGCCGCCATCGTCATCGGAGCTCAGGC
2-3  GGCACGAGCAAAAAATGAAACTCCTCTTGTGCTTTGCTTTCGCCGCCATCGTCATCGGAGCTCAGGC
3-4  GGCACGAGCAAAAAATGAAACTCCTCTTGTGCTTTGCTTTCGCCGCCATCGTCATCGGAGCTCAGGC
3-9  GGCACGAGCAAAAAATGAAACTCCTCTTGTGCTTTGCTTTCGCCGCCATCGTCATCGGAGCTCAGGC
7-5  GGCACGAGCAAAAAATGAAACTCCTCTTGTGCTTTGCGTTCGCCGCCATCGTCATCGGAGCTCAGGC

2-2  TCTCACCGACGAACAGATACAGAAAAGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGT
2-3  TCTCACCGACGAACAGATACAGAAAAGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGT
3-4  TCTCACCGACGAACAGATACAGAAAAGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGT
3-9  TCTCACCGATGAACAGATACAGAAAAGGAACAAGATCAGCAAAGAATGCCAGCAGGAGTCCGGAGT
7-5  TCTCACCGACGAACAGATACAGAAAAGGAACAAGATCAGCAAAGAGTGCCAGCAGGTGTCCGGAGT

2-2  GTCCCAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGATGATCCCAAAATGAAGAAGCA
2-3  GTCCCAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGATCCCAAAATGAAGAAGCA
3-4  GTCCCAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGATCCCAAAATGAAGAAGCA
3-9  GTCCCAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGATCCCAAAATGAAGAAGCA
7-5  GTCCCAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGATCCCAAAATGAAGAAGCA

2-2  CGTCCTCTGCTTCTCGAAGAAAACTGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACT
2-3  CGTCCTCTGCTTCTCGAAGAAAACTGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACT
3-4  CGTCCTCTGCTTCTCGAAGAAAACTGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACT
3-9  CGTCCTCTGCTTCTCGAAGAGAACTGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACT
7-5  CGTCCTCTGCTTCTCGAAGAAAACTGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACT

2-2  CAAAGCCAAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTGCAGAAGTGCGTGGT
2-3  CAAAGCCAAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTGCAGAAGTGCGTGGT
3-4  CAAAGCCAAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTGCAGAAGTGCGTGGT
3-9  CAAAGCCAAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTGCAGAAGTGCGTGGT
7-5  CAAAGCCAAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTGCAGAAGTGCGTGGT

2-2  CAAGAAGGCCACACCAGAGGAAACGGCTTATGACACCTTCAAGTGTATTTACGACAGCAAACCTGA
2-3  CAAGAAGGCCACACCAGAGGAAACGGCTTATGACACCTTCAAGTGTATTTACGACAGTAAACCTGA
3-4  CAAGAAGGCCACACCAGAGGAAACGGCTTATGACACCTTCAAGGTTATTTACGACAGTAAACCTGA
3-9  CAAGAAGGCCACACCAGAGGAAACGGCTTATGACACCTTCAAGTGTATTTACGACAGTAAACCTGA
7-5  CAAGAAGGCCACACCAGAGGAAACGGCTTATGACACCTTCAAGTGTATTTACGACAGTAAACCTGA

                      *
2-2  TTTCTCTCCTATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGTAATATCGTTAT
2-3  TTTCTCTCCTATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGTACTATCGTTAT
3-4  TTTCTCTCCTATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGTACTATCGTTAT
3-9  TTTCTCTCCTATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGTACTATCGTTAT
7-5  TTTCTCTCCTATTGATTAATTGTTTTGTATTTGGCTGAATTTTGACAATAAAGGTACTATCGTTAT

2-2  GT AAAAAAAAAAAAAAAAAAA
2-3  GAAAAAAAAAAAAAAAAAAAAA
3-4  GT AAAAAAAAAAAAAAAAAAA
3-9  GAAAAAAAAAAAAAAAAAAAAA
7-5  GT AAAAAAAAAAAAAAAAAAA
```

FIG. 4.13

```
          ↓
2-2 MKLLLCFAFAAI VI GAQALTDEQI QKRNKI SKECQQVSGVSQETI DKVRTGVLV
2-3 MKLLLCFAFAAI VI GAQALTDEQI QKRNKI SKECQQVSGVSQETI DKVRTGVLV
3-4 MKLLLCFAFAAI VI GAQALTDEQI QKRNKI SKECQQESGVSQETI DKVRTGVLV
3-9 MKLLLCFAFAAI VI GAQALTDEQI QKRNKI SKECQQVSGVSQETI DKVRTGVLV
7-5 MKLLLCFAFAAI VI GAQALTDEQI QKRNKI SKECQQVSGVSQETI DKVRTGVLV

2-2 DDPKMKKHVLCFSKKTGVATEAGDTNVEVLKAKLKHVASDEEVDKI VQKCVVKK
2-3 DDPKMKKHVLCFSKKTGVATEAGDTNVEVLKAKLKHVASDEEVDKI VQKCVVKK
3-4 DDPKMKKHVLCFSKKTGVATEAGDTNVEVLKAKLKHVASDEEVDKI VQKCVVKK
3-9 DDPKMKKHVLCFSKRTGVATEAGDTNVEVLKAKLKHVASDEEVDKI VQKCVVKK
7-5 DDPKMKKHVLCFSKKTGVATEAGDTNVEVLKAKLKHVASDEEVDKI VQKCVVKK

2-2 ATPEETAYDTFKCI YDSKPDFSPI D*
2-3 ATPEETAYDTFKCI YDSKPDFSPI D*
3-4 ATPEETAYDTFKVI YDSKPDFSPI D*
3-9 ATPEETAYDTFKCI YDSKPDFSPI D*
7-5 ATPEETAYDTFKCI YDSKPDFSPI D*
```

FIG. 4.14

(% mole)

	MW (kDa)	AA	Cys	Pro	Phe	Ile	Val	Met	Leu	% most hydrophobic	Gly	Ala	Tyr	His	Trp	Asx	Glx	Arg	Lys	Ser	Thr	% most hydrophilic
Tm 12.86	12.86	117	3.2	3.0	3.4	4.4	8.5	2.0	4.4	28.9	3.1	3.9	3.8	3.2	ND	10.7	15.0	3.6	14.9	6.8	6.3	57.3
Tm 13.17	13.17	116	3.13	2.21	4.47	5.16	10.5	1.0	4.3	29.11	1.73	3.24	0	0	1.41	0	0	7.12	15.6	3.31	6.14	32.14
2-2	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.08	32.23
2-3	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.08	32.23
3-4	12.84	115	2.41	3.02	3.44	5.29	11.6	1.02	4.41	28.04	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.09	32.24
3-9	12.87	115	3.21	3.02	3.43	5.28	10.8	1.02	4.40	27.20	1.77	3.31	2.54	2.13	0	0	0	3.64	16.9	4.74	7.07	32.38
7-5	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.08	32.23

FIG. 4.15

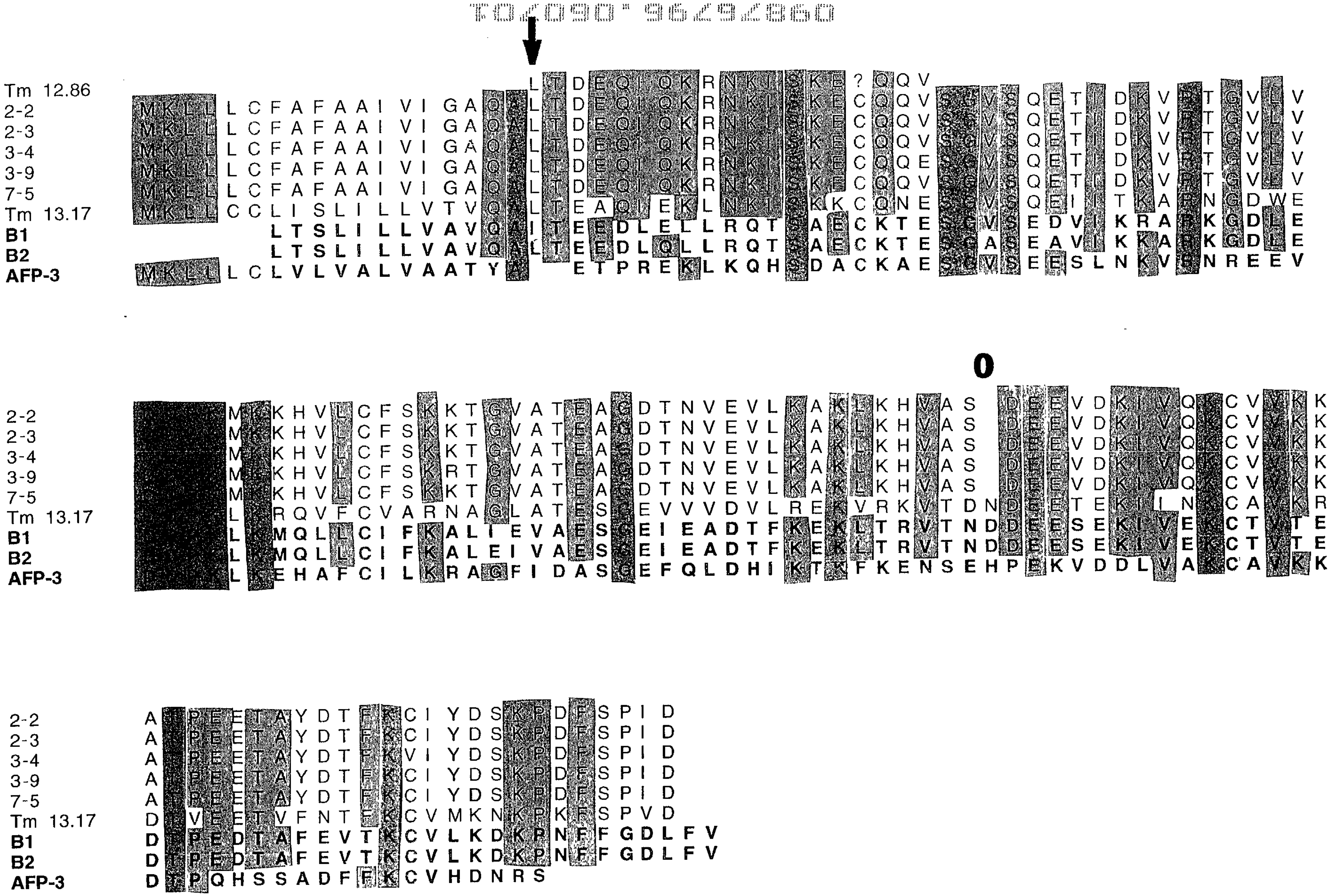


FIG. 4.16

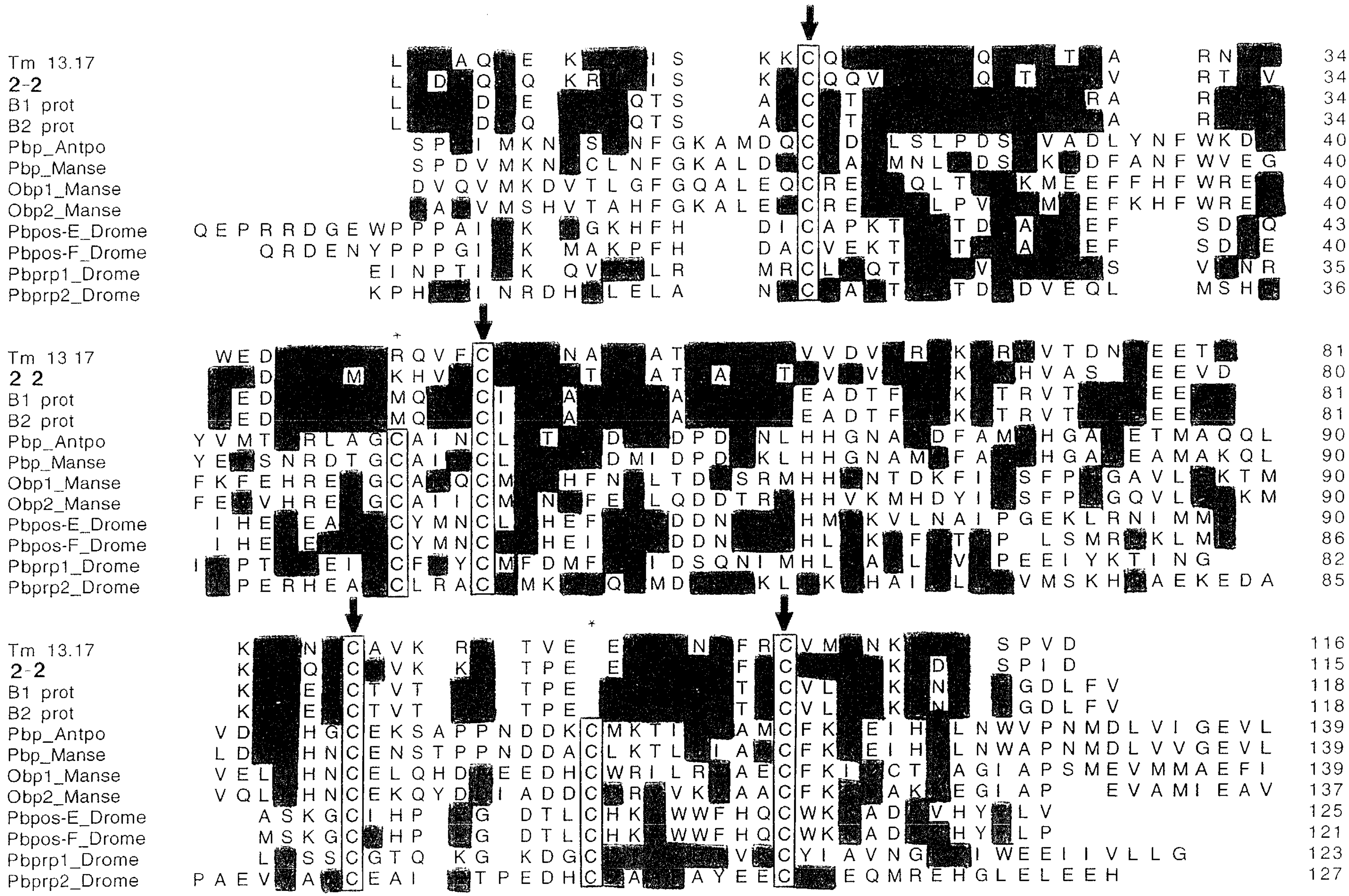

Tm 13.17
2-2
B1 prot
B2 prot
Pbp_Antpo
Pbp_Manse
Obp1_Manse
Obp2_Manse
Pbpos-E_Drome
Pbpos-F_Drome
Pbprp1_Drome
Pbprp2_Drome

FIG. 4.17

Tm 12.86 L T D E Q I Q K R N [redacted] I S [redacted] E ? Q Q V
2-2 M [redacted] L L L C F A F A A I V I G A Q A L T D E Q I Q K R N [redacted] I S [redacted] E C Q Q V S G V S Q E T I D K V R T G V L V
2-3 M [redacted] L L L C F A F A A I V I G A Q A L T D E Q I Q K R N [redacted] I S [redacted] E C Q Q V S G V S Q E T I D K V R T G V L V
3-4 M [redacted] L L L C F A F A A I V I G A Q A L T D E Q I Q K R N [redacted] I S [redacted] E C Q Q V S G V S Q E T I D K V R T G V L V
3-9 M [redacted] L L L C F A F A A I V I G A Q A L T D E Q I Q K R N [redacted] I S [redacted] E C Q Q E S G V S Q E T I D K V R T G V L V
7-5 M [redacted] L L L C F A F A A I V I G A Q A L T D E Q I Q K R N [redacted] I S [redacted] E C Q Q V S G V S Q E T I D K V R T G V L V
Tm 13.17 M [redacted] L L C C L I S L I L L V T V Q A L T E A Q I E K L N [redacted] I S [redacted] K C Q N E S G V S Q E I I T K A R N G D W E
B1 **L T S L I L L V A V Q A I T E E D L E L L R Q T S A E C K T E S G V S E D V I K R A R K G D L E**
B2 **L T S L I L L V A V Q A L T E E D L Q L L R Q T S A E C K T E S G A S E A V I K K A R K G D L E**
AFP-3 M [redacted] L L L C **L V L V A L V A A T Y A E T P R E K L K Q H S D A C K A E S G V S E E S L N K V R N R E E V**

2-2 D D P K M [redacted] H V L C F S K K T G V A T E A G D T N V E V L K A K L K H V A S D E E V D [redacted] I V Q [redacted] C V V K K
2-3 D D P K M [redacted] H V L C F S K K T G V A T E A G D T N V E V L K A K L K H V A S D E E V D [redacted] I V Q [redacted] C V V K K
3-4 D D P K M [redacted] H V L C F S K K T G V A T E A G D T N V E V L K A K L K H V A S D E E V D [redacted] I V Q [redacted] C V V K K
3-9 D D P K M [redacted] H V L C F S K R T G V A T E A G D T N V E V L K A K L K H V A S D E E V D [redacted] I V Q [redacted] C V V K K
7-5 D D P K M [redacted] H V L C F S K K T G V A T E A G D T N V E V L K A K L K H V A S D E E V D [redacted] I V Q [redacted] C V V K K
Tm 13.17 D D P K L [redacted] R Q V F C V A R N A G L A T E S G E V V V D V L R E K V R K V T D N D E E T E [redacted] I I N [redacted] C A V K R
B1 **D D P K L** [redacted] **M Q L L C I F K A L I E V A E S G E I E A D T F K E K L T R V T N D D E E S E** [redacted] **I V E** [redacted] **C T V T E**
B2 **D D P K L** [redacted] **M Q L L C I F K A L E I V A E S G E I E A D T F K E K L T R V T N D D E E S E** [redacted] **I V E** [redacted] **C T V T E**
AFP-3 **D D P K L** [redacted] **E H A F C I L K R A G F I D A S G E F Q L D H I K T K F K E N S E H P E K V D D L V A** [redacted] **C A V K K**

2-2 A T P E E T A Y D T F [redacted] C I Y D S K P D F S P I D
2-3 A T P E E T A Y D T F [redacted] C I Y D S K P D F S P I D
3-4 A T P E E T A Y D T F [redacted] V I Y D S K P D F S P I D
3-9 A T P E E T A Y D T F [redacted] C I Y D S K P D F S P I D
7-5 A T P E E T A Y D T F [redacted] C I Y D S K P D F S P I D
Tm 13.17 D T V E E T V F N T F [redacted] C V M K N K P K F S P V D
B1 **D T P E D T A F E V T** [redacted] **C V L K D K P N F F G D L F V**
B2 **D T P E D T A F E V T** [redacted] **C V L K D K P N F F G D L F V**
AFP-3 **D T P Q H S S A D F F** [redacted] **C V H D N R S**

FIG. 4.18

NUCLEOTIDE SEQUENCES

PERCENT SIMILARITY (upper right); PERCENT DIVERGENCE (lower left)

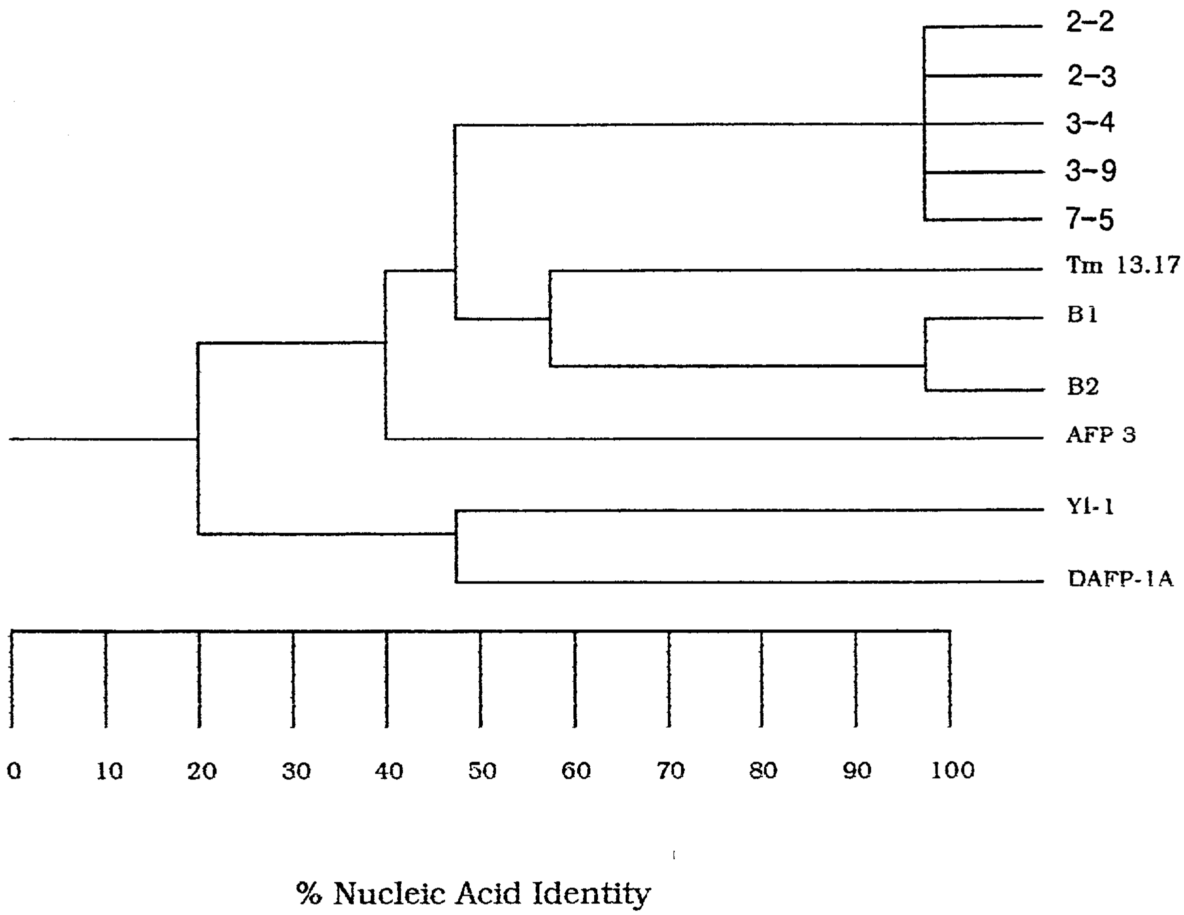
	1 (2-2)	2 (2-3)	3 (3-4)	4 (3-9)	5 (7-5)	6 (Tm13.17)	7 (B1)	8 (AFP-3)	9 (YL-1)	10 (DAFP-1A)		
1		99.1	97.3	98.4	98.2	50.4	42	42.4	20.7	20.9	1	2-2
2	0.9		99	99	99	51.5	43.2	42.7	20.2	21.3	2	2-3
3	1.6	60		98	98	50.3	42	42.1	20	20	3	3-4
4	1.6	20	80		98.4	51.3	43.7	43.6	20.4	22	4	3-9
5	1.8	25	75	45		50.4	42.5	42.9	22.2	23.1	5	7-5
6	36.9	36.4	37.2	36.4	37.1		57.2	37.4	23.3	21	6	Tm 13.17
7	39.9	41.2	41.7	40.7	41.2	30.2		39.3	21.9	22.1	7	B1
8	41.8	42.4	43.1	42.3	42.3	45.3	49.4		21.8	23.1	8	AFP-3
9	48.7	58.6	52	51.2	51.9	61.7	62.2	58.2		45.6	9	YL-1
10	51.4	62.7	46.4	46.5	46.5	60	58.1	61.8	35.5		10	DAFP-1A
	1	2	3	4	5	6	7	8	9	10		

AMINO ACID SEQUENCES

PERCENT SIMILARITY (upper right); PERCENT DIVERGENCE (lower left)

	1 (2-2)	2 (2-3)	3 (3-4)	4 (3-9)	5 (7-5)	6 (Tm13.17)	7 (B1)	8 (AFP-3)	9 (YL-1)	10 (DAFP-1A)		
1		100	99.1	99.1	100	51.3	37.4	35.2	11.6	12	1	2-2
2	0		99.1	99.1	100	51.3	37.4	35.2	11.6	12	2	2-3
3	0.9	0.9		98.3	99.1	50.4	36.5	34.3	11.6	12	3	3-4
4	0.9	0.9	1.7		99.1	51.3	37.4	36.1	10.7	12	4	3-9
5	0	0	0.9	0.09		51.3	37.4	35.2	11.6	12	5	7-5
6	46.1	46.1	47	46.1	46.1		47.4	39.8	13.4	13.9	6	Tm 13.17
7	59.1	59.1	60	59.1	59.1	51.7		37	11.6	11.1	7	B1
8	61.7	61.7	62.6	60.7	61.7	60.2	63		10.2	8.3	8	AFP-3
9	86.7	86.7	85.7	85.7	85.7	86.8	84.2	87.5		55.6	9	YL-1
10	88.3	88.3	86.4	84.5	85.4	89.1	82.7	90.5	40.2		10	DAFP-1A
	1	2	3	4	5	6	7	8	9	10		

FIG 4.19



2-2
2-3
3-4
3-9
7-5
Tm 13.17
B1
B2
AFP 3
Yl-1
DAFP-1A
0
10
20
30
40
50
60
70
80
90
100
% Nucleic Acid Identity

FIG 4.20

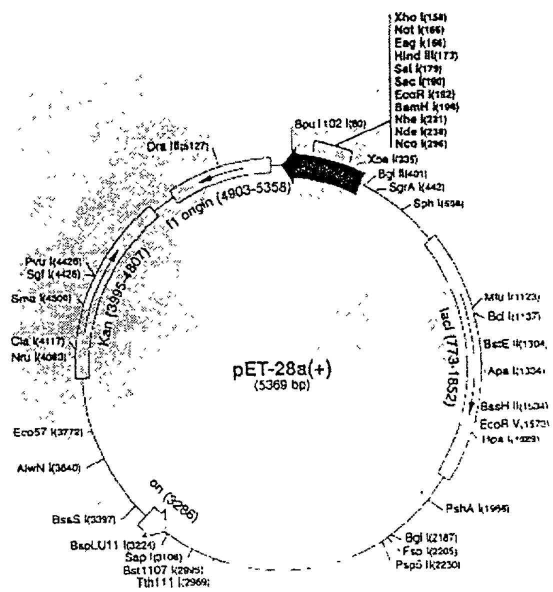
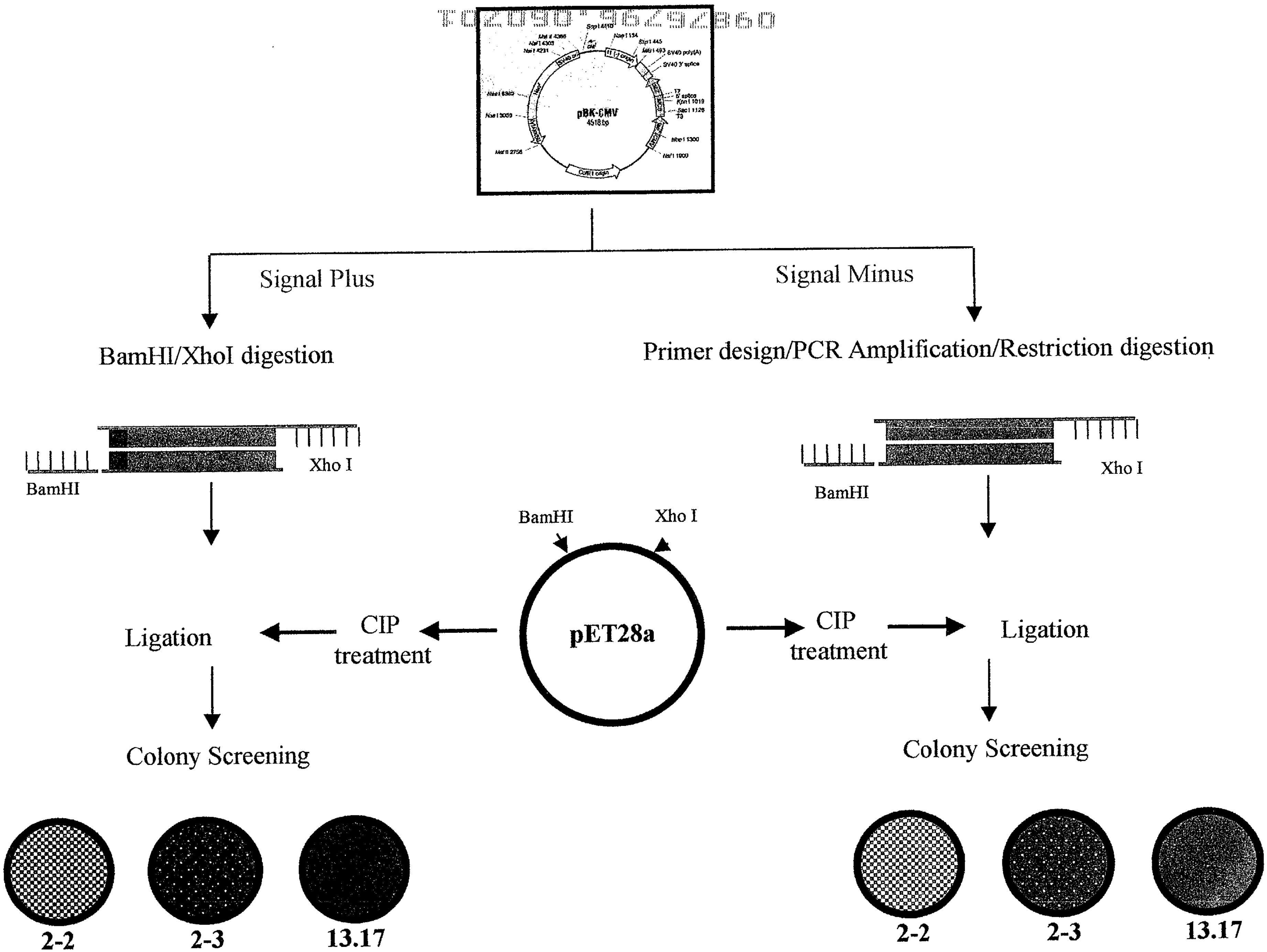

pET-28a(+)
(5369 bp)
f1 origin (4903-5358)
Kan (3995-4807)
lacI (773-1852)
ori (3286)

FIG. 5.0



pBK-CMV
4518 bp
Signal Plus
Signal Minus
BamHI/XhoI digestion
Primer design/PCR Amplification/Restriction digestion
BamHI
Xho I
BamHI
Xho I
BamHI
Xho I
pET28a
CIP
treatment
CIP
treatment
Ligation
Ligation
Colony Screening
Colony Screening
2-2
2-3
13.17
2-2
2-3
13.17

FIG. 5.1

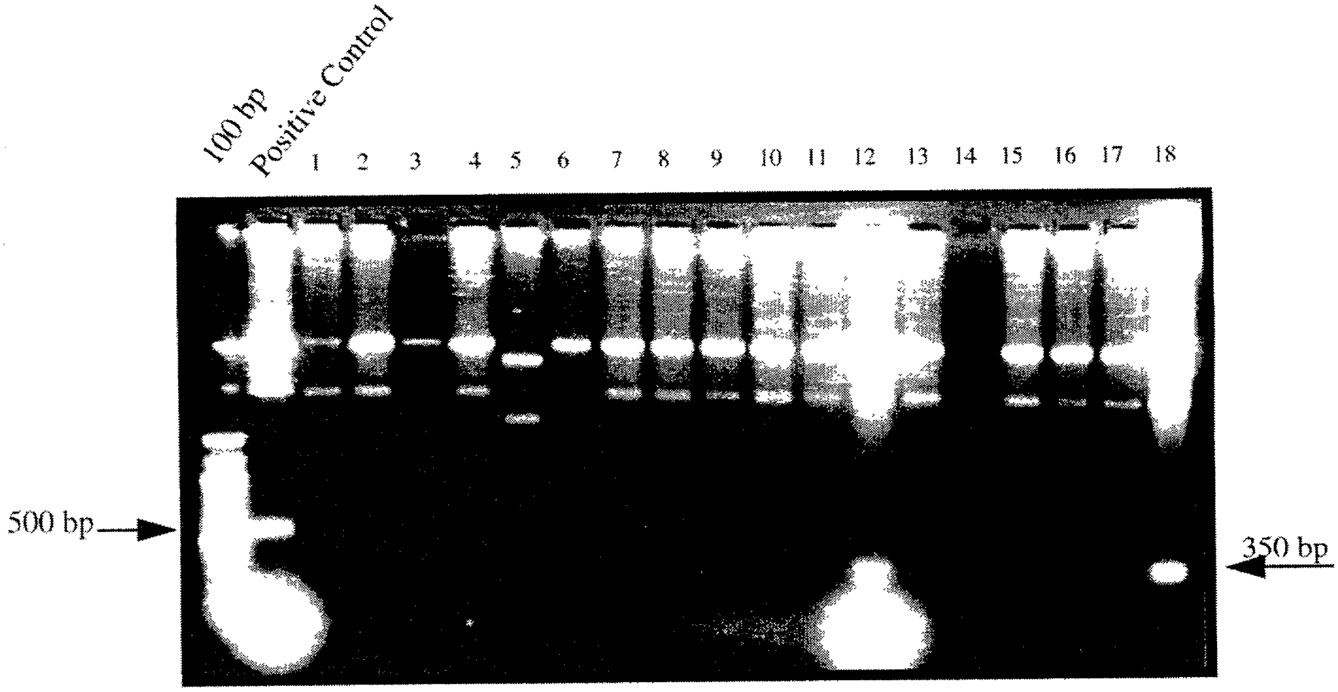
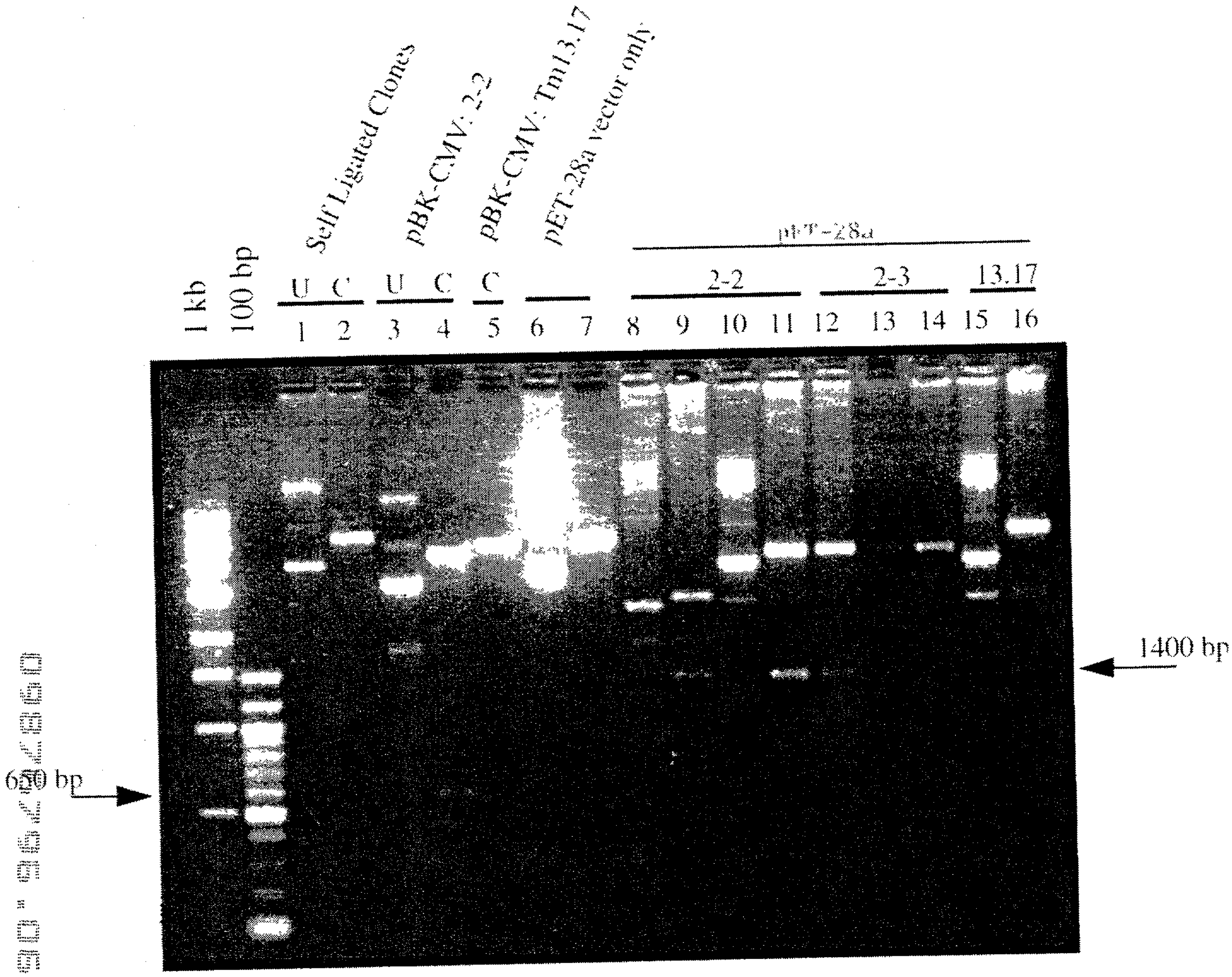
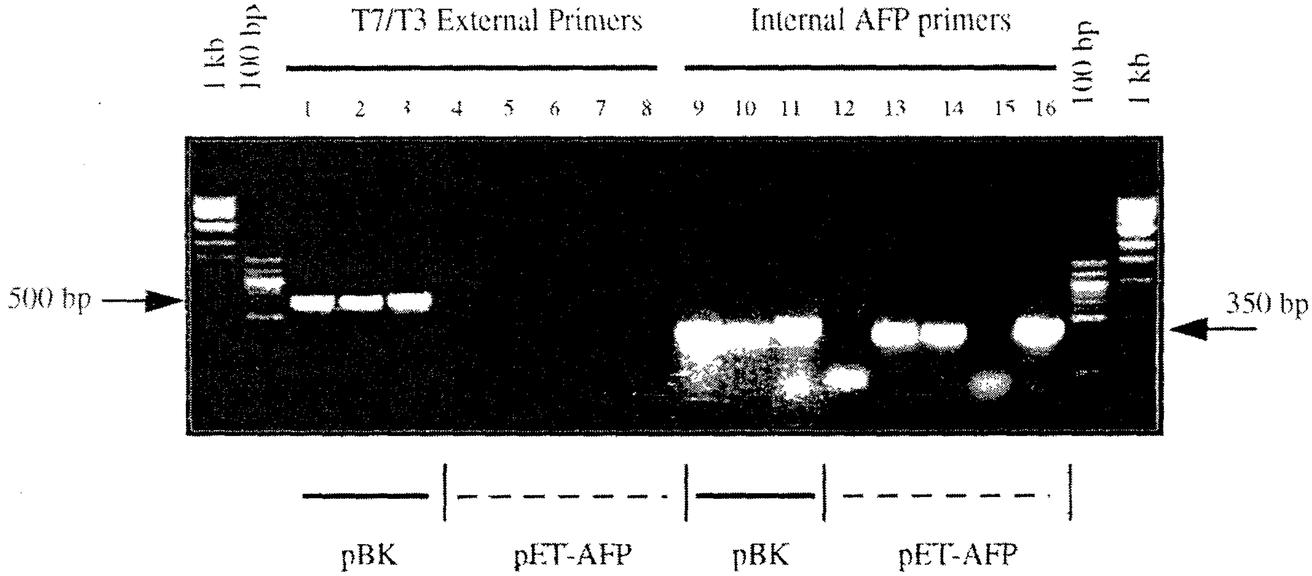

100 bp
Positive Control
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
500 bp
350 bp

FIG. 5.2



1 kb
100 bp
Self Ligated Clones
pBK-CMV: 2-2
pBK-CMV: Tm13.17
pET-28a vector only
pET-28a
U C U C C
2-2
2-3
13.17
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
650 bp
1400 bp

FIG. 5.3



1 kb
100 bp
T7/T3 External Primers
Internal AFP primers
100 bp
1 kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
500 bp
350 bp
pBK
pET-AFP
pBK
pET-AFP

FIG. 5.4

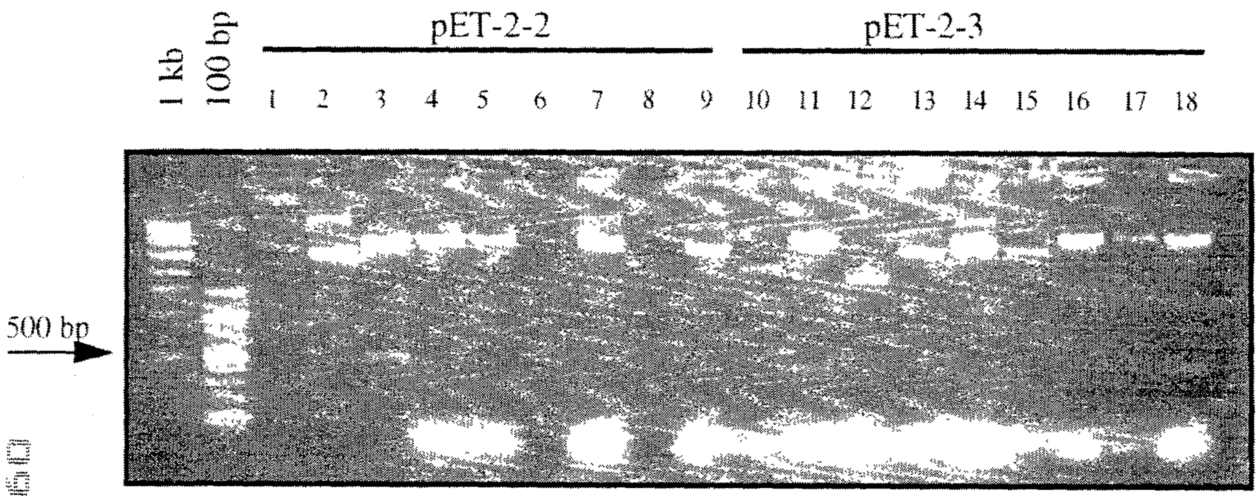

pET-2-2
pET-2-3
1 kb
100 bp
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
500 bp

FIG. 5.5

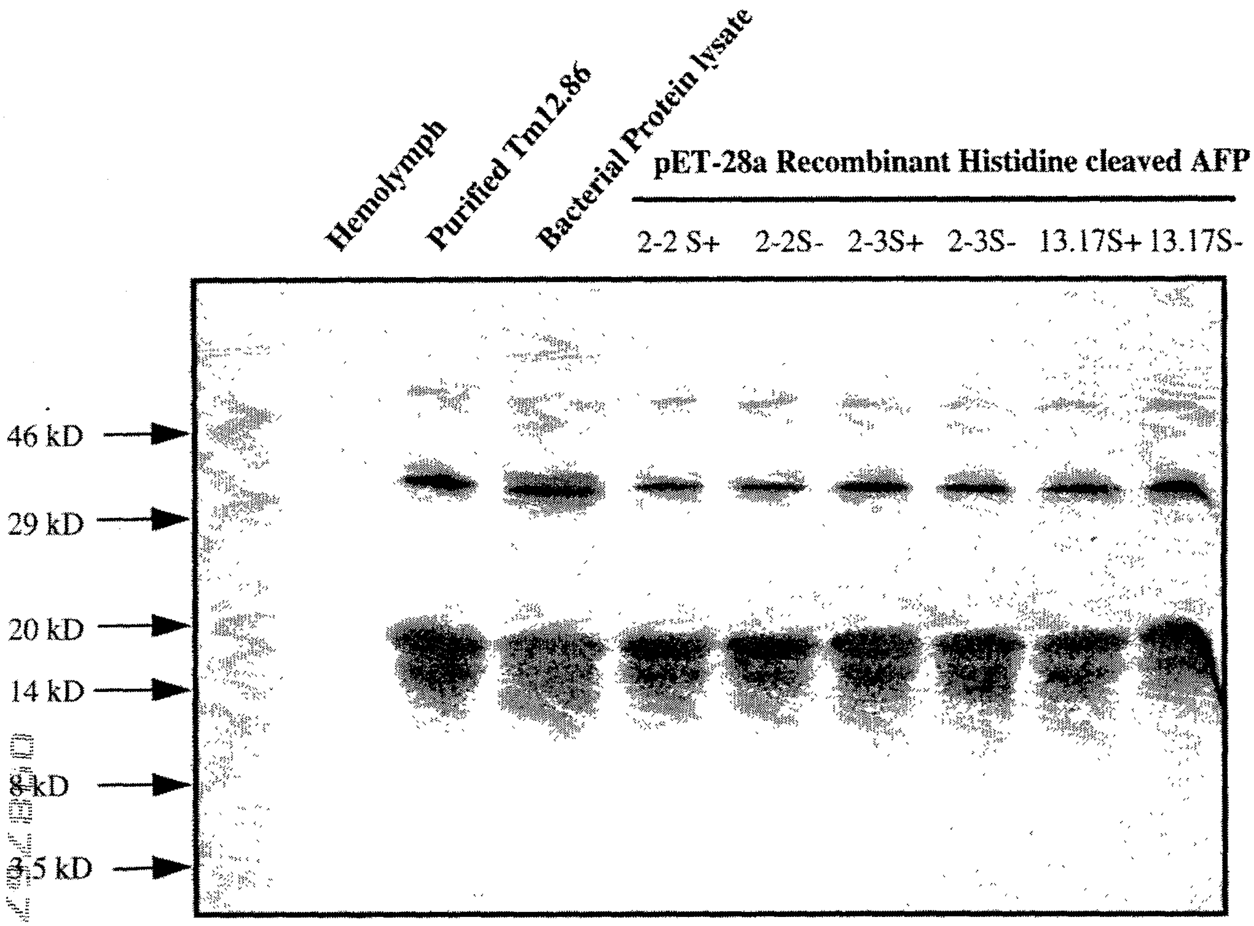

Hemolymph
Purified Tm12.86
Bacterial Protein lysate
pET-28a Recombinant Histidine cleaved AFP
2-2 S+
2-2S-
2-3S+
2-3S-
13.17S+
13.17S-
46 kD
29 kD
20 kD
14 kD
8 kD
3.5 kD

FIG. 5.6

His-tagged Clone 2.2 with signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG           50

               His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC       96
               Met Gly Ser Ser His His His His His His Ser
                           -55                   -50

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT     141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
        -45                 -40                 -35

                                                    AFP Start Codon
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG     186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys Met
        -30                 -25                 -20

AAA CTC CTC TTG TGC TTT GCG TTC GCC GCC ATC GTC ATC GGA GCT     231
Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala
        -15                 -10                 -5

        N-terminal of mature AFP
CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC     276
Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser
        1                   5                   10

AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC     321
Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp
    15                  20                  25

AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG     366
Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys
    30                  35                  40

CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC     411
His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala
    45                  50                  55

GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG     456
Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val
    60                  65                  70

GCC AGC GAC GAA GAG GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC     501
Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val
    75                  80                  85

AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT     546
Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys
    90                  95                  100

                                                Stop Codon
ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTTGTA  595
Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp *
    105                 110                 115

                  Polyadenylation signal    Poly-A tail
TTTGACTGAA TTTTGACAAT AAAGGTAATA TCGTTATGTA AAAAAAAAAA           645

AAAAAACTCG AGCACCACCA CCACCACCAC TGAGAT                          681
```

FIG. 5.7

His-tagged clone 2.2 without signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG            50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC         96
               Met Gly Ser Ser His His His His His His Ser
                               -30                 -25

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT       141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
            -20                 -15                 -10

                                N-terminal of mature AFP
GGA CAG CAA ATG GGT CGC GGA TCC CTC ACC GAC GAA CAG ATA CAG       186
Gly Gln Gln Met Gly Arg Gly Ser Leu Thr Asp Glu Gln Ile Gln
            -5                  1               5

AAA AGG AAC AAG ATC AGC AAA GAA TGC CAG CAG GTG TCC GGA GTG       231
Lys Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val
        10                  15                  20

TCC CAA GAG ACG ATC GAC AAA GTC CGC ACA GGT GTC TTG GTC GAT       276
Ser Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp
        25                  30                  35

GAT CCC AAA ATG AAG AAG CAC GTC CTC TGC TTC TCG AAG AAA ACT       321
Asp Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr
        40                  45                  50

GGA GTG GCA ACC GAA GCC GGA GAC ACC AAT GTG GAG GTA CTC AAA       366
Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys
        55                  60                  65

GCC AAG CTG AAG CAT GTG GCC AGC GAC GAA GAG GTG GAC AAG ATC       411
Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile
        70                  75                  80

GTG CAG AAG TGC GTG GTC AAG AAG GCC ACA CCA GAG GAA ACG GCT       456
Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala
        85                  90                  95

TAT GAC ACC TTC AAG TGT ATT TAC GAC AGT AAA CCT GAT TTC TCT       501
Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser
        100                 105                 110

            Stop Codon
CCT ATT GAT TAA CTCGAGCACC ACCACCACCA CCACTGAGAT                  543
Pro Ile Asp *
        115
```

FIG. 5.8

His-tagged clone 2.3 with signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC            96
               Met Gly Ser Ser His His His His His His Ser
                           -55                      -50

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT         141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
    -45                     -40                     -35

                                                        AFP Start Codon
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG         186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys Met
    -30                     -25                     -20

AAA CTC CTC TTG TGC TTT GCT TTC GCC GCC ATC GTC ATC GGA GCT         231
Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala
    -15                     -10                     -5

    N-terminal of Mature AFP
CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC         276
Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser
        1               5                   10

AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC         321
Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp
    15                  20                  25

AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG         366
Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys
    30                  35                  40

CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC         411
His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala
    45                  50                  55

GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG         456
Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val
    60                  65                  70

GCC AGC GAC GAA GAA GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC         501
Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val
    75                  80                  85

AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT         546
Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys
    90                  95                  100

                                                Stop Codon
ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTGTA       595
Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp *
    105                 110                 115

                      Polyadenylation signal    Poly-A tail
TTTGACTGAA TTTTGACAAT AAAGGTACTA TCGTTATGAA AAAAAAAAA               645

AAAAAAACTC GAGCACCACC ACCACCACCA CTGAGAT                             682
```

FIG. 5.9

His-tagged Clone 2.3 without signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG         50

               His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC     96
               Met Gly Ser Ser His His His His His His Ser
                               -30                 -25

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT    141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
            -20                 -15                 -10

                                N-terminal of mature AFP
GGA CAG CAA ATG GGT CGC GGA TCC CTC ACC GAC GAA CAG ATA CAG    186
Gly Gln Gln Met Gly Arg Gly Ser Leu Thr Asp Glu Gln Ile Gln
            -5                  1               5

AAA AGG AAC AAG ATC AGC AAA GAA TGC CAG CAG GTG TCC GGA GTG    231
Lys Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val
        10                  15                  20

TCC CAA GAG ACG ATC GAC AAA GTC CGC ACA GGT GTC TTG GTC GAT    276
Ser Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp
        25                  30                  35

GAT CCC AAA ATG AAG AAG CAC GTC CTC TGC TTC TCG AAG AAA ACT    321
Asp Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr
        40                  45                  50

GGA GTG GCA ACC GAA GCC GGA GAC ACC AAT GTG GAG GTA CTC AAA    366
Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys
        55                  60                  65

GCC AAG CTG AAG CAT GTG GCC AGC GAC GAA GAA GTG GAC AAG ATC    411
Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile
        70                  75                  80

GTG CAG AAG TGC GTG GTC AAG AAG GCC ACA CCA GAG GAA ACG GCT    456
Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala
        85                  90                  95

TAT GAC ACC TTC AAG TGT ATT TAC GAC AGT AAA CCT GAT TTC TCT    501
Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser
        100                 105                 110

            Stop Codon
CCT ATT GAT TAA CTCGAGCACC ACCACCACCA CCACTGAGAT               543
Pro Ile Asp *
        115
```

FIG. 5.10

His-tagged Tm 13.17 with signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG           50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC       96
               Met Gly Ser Ser His His His His His His Ser
               -65                 -60                 -55

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT     141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
                -50                 -45                 -40

GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC TGG ATC CAA AGA ATT     186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Trp Ile Gln Arg Ile
                -35                 -30                 -25

                         AFP Start Codon
CGG CAC GAG ACT ACT AAG ATG AAG TTG CTC TGT TGT CTA ATC TCC     231
Arg His Glu Thr Thr Lys Met Lys Leu Leu Cys Cys Leu Ile Ser
                -20                 -15                 -10

                                    N-terminal of mature AFP
CTC ATT CTG TTG GTC ACA GTT CAG GCC CTG ACC GAG GCA CAA ATT     276
Leu Ile Leu Leu Val Thr Val Gln Ala Leu Thr Glu Ala Gln Ile
                -5                  1               5

GAG AAA CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA     321
Glu Lys Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly
            10                  15                  20

GTG TCG CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG     366
Val Ser Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu
            25                  30                  35

GAC GAT CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC     411
Asp Asp Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn
            40                  45                  50

GCC GGT CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG     456
Ala Gly Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu
            55                  60                  65

AGG GAG AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG     501
Arg Glu Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu
            70                  75                  80

AAA ATC ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG     546
Lys Ile Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu
            85                  90                  95

ACG GTG TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG     595
Thr Val Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys
            100                 105                 110

                    Stop Codon
TTC TCA CCA GTT GAT TGA ACCACCACGA CTAGTAGATG GTTCAAATGG        643
Phe Ser Pro Val Asp *
            115

                   Polyadenylation signal  Poly-A tail
TGTGCTTTAC ATATAAAAAT AAAGTGTTTC TGATGTAAAA AAAAAAAAAA          693

AAAAAAAAAA AACTCGAGAG TATTCTAGAG CGGCCGCGGG CCCATCGTTT          743

TCCACCCCTC GAGCACCACC ACCACCACCA CTGAGAT                        777
```

FIG. 5.11

His-tagged Tm 13.17 without signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG              50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC          96
               Met Gly Ser Ser His His His His His His Ser
                       -30                      -25

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT        141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
    -20                     -15                 -10

                            N-terminal of mature AFP
GGA CAG CAA ATG GGT CGC GGC CTG ACC GAG GCA CAA ATT GAG AAA        186
Gly Gln Gln Met Gly Arg Gly Leu Thr Glu Ala Gln Ile Glu Lys
    -5                      1               5

CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA GTG TCG        231
Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly Val Ser
    10                  15                  20

CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG GAC GAT        276
Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu Asp Asp
    25                  30                  35

CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC GCC GGT        321
Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn Ala Gly
    40                  45                  50

CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG AGG GAG        366
Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu Arg Glu
    55                  60                 65

AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG AAA ATC        411
Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu Lys Ile
    70                  75                  80

ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG ACG GTG        456
Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu Thr Val
    85                  90                  95

TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG TTC TCA        501
Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys Phe Ser
    100                 105                 110

            Stop Codon
CCA GTT GAT TGA CTCGAGCACC ACCACCACCA CCACTGAGAT                   543
Pro Val Asp *
    115
```

FIG. 5.12

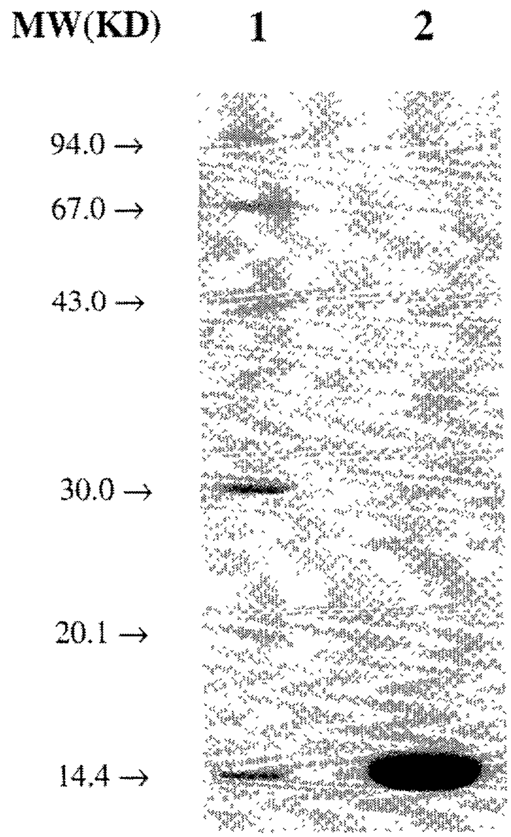


FIG. 6.0

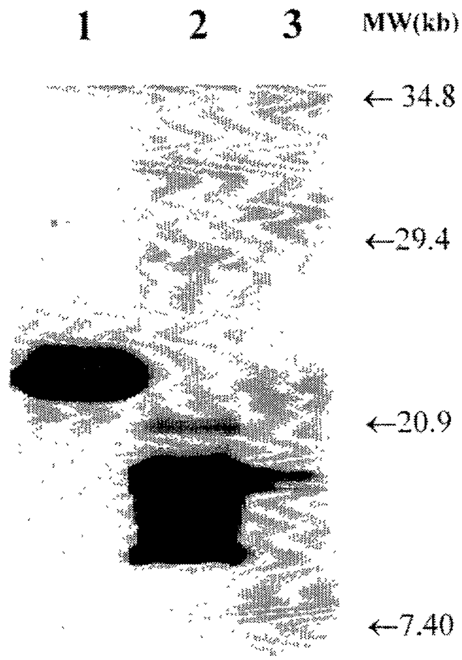


FIG. 6.1

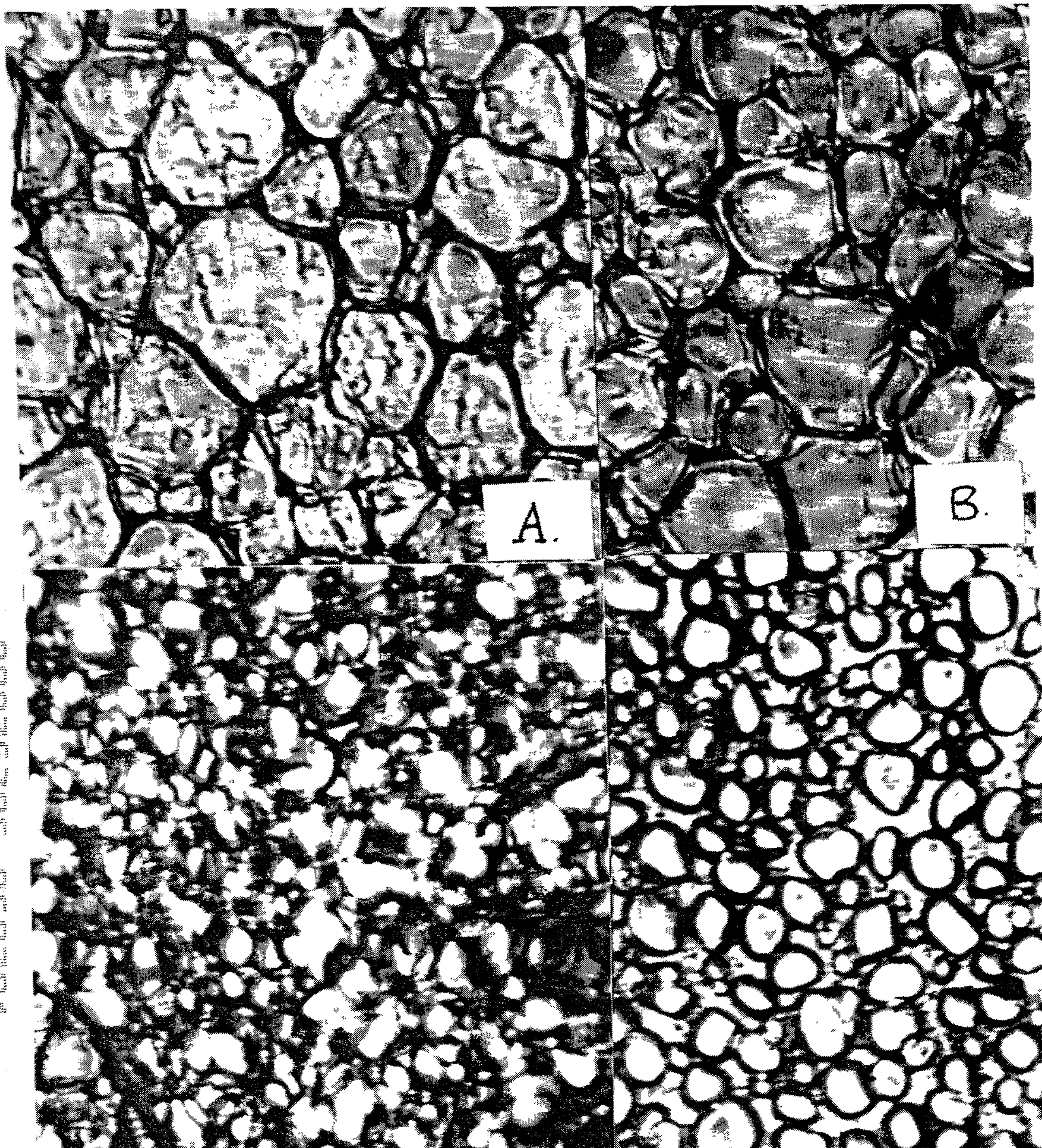


FIG. 6.2

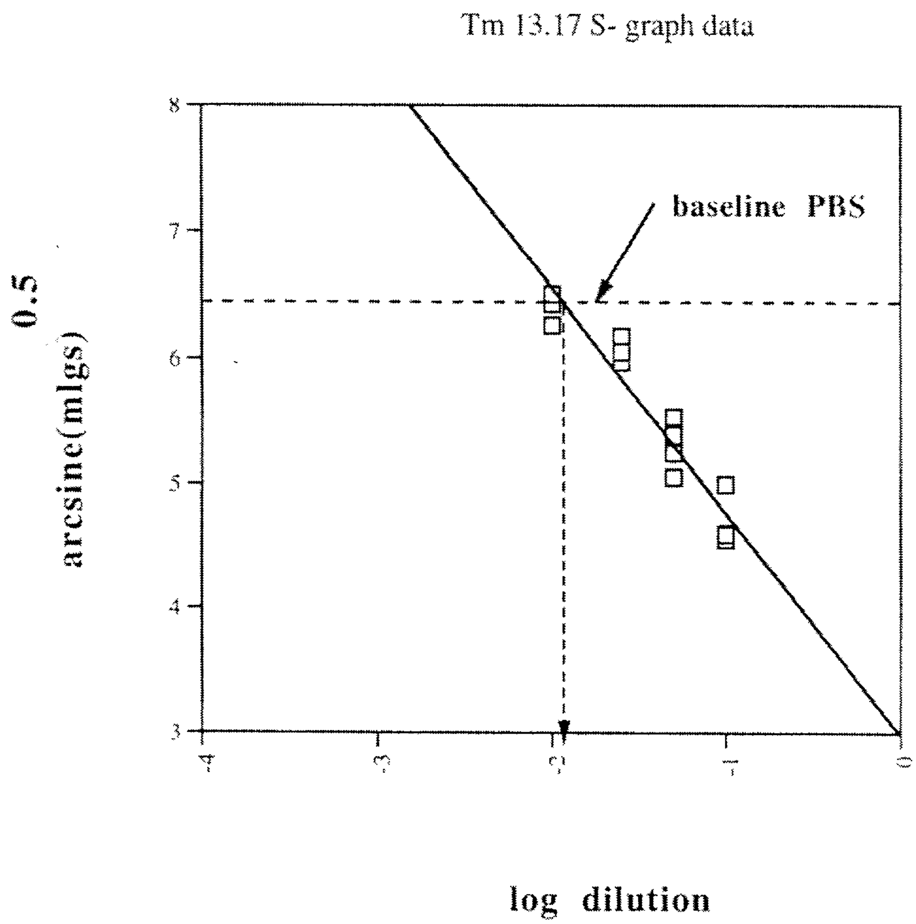

Tm 13.17 S- graph data
baseline PBS
8
7
6
5
4
3
-4
-3
-2
-1
0
0.5
arcsine(mlgs)
log dilution

FIG. 6.3

ONE LETTER	NAME	THREE LETTER	CHEMICAL CLASS	HYDROPHOBICITY	Reactivity
A	Alanine	Ala	aliphatic	mod. hydrophobic	low
B	Asp or Asn	Asx			
C	Cysteine	Cys	sulfhydryl	hydrophobic	high
D	Aspartic Acid	Asp	acidic	highly hydrophilic	high
E	Glutamic Acid	Glu	acidic	highly hydrophilic	high
F	Phenylalanine	Phe	aromatic	highly hydrophobic	low
G	Glycine	Gly	aliphatic	mod. hydrophobic	low
H	Histidine	His	basic, imidazole	highly hydrophilic	high
I	Isoleucine	Ile	aliphatic	hydrophobic	low
J					
K	Lysine	Lys	basic	highly hydophilic	high
L	Leucine	Leu	aliphatic	hydrophobic	low
M	Methionine	Met	sulfhydryl	hydrophobic	low
N	Asparagine	Asn	amide, acidic derived	hydrophilic	high
O					
P	Proline	Pro	aliphatic, cyclic, imino	mod. hydrophilic	low
Q	Glutamine	Gln	amide, acidic derived	hydrophilic	high
R	Arginine	Arg	basic	highly hydrophilic	high
S	Serine	Ser	aliphatic hydroxyl	hydrophilic	high
T	Threonine	Thr	aliphatic hydroxyl	hydrophilic	high
U					
V	Valine	Val	aliphatic	hydrophobic	low
W	Tryptophan	Trp	aromatic	highly hydrophobic	low
X					
Y	Tyrosine	Tyr	aromatic	mod. hydrophilic	high
Z	Glu or Gln	Glx			
		ACD	Any Acidic		
		ALP	Any Aliphatic		
		ALH	Any Aliphatic Hydroxyl		
		ARO	Any Aromatic		
		BAS	Any Basic		
		HY-	Hydrophobic		
		HY+	Hydrophilic		

FIG. 7.1

Position	Tm 12 84-2.2	Tm 12 84-2 3	Tm 12.84-3 4	Tm 12 84-3 9	Tm 12.84-7 5	Concensus of Tm 12 84	Tm13 17	Concensus with Tm 13.17	B1	Concensus with B1	AFP-3	Concensus with AFP-3
1	A	A	A	A	A	A	A	A		A	C	N
2	C	C	C	C	C	C	G	N		N	A	N
3	G	G	G	G	G	G	A	R		R	G	R
4	A	A	A	A	A	A	C	N		N	A	N
5	G	G	G	G	G	G	T	N		N	T	N
6	C	C	C	C	C	C	A	N		N	C	N
7	A	A	A	A	A	A	C	N		N	C	N
8	A	A	A	A	A	A	T	N		N	G	N
9	A	A	A	A	A	A	A	A		A	A	A
10	A	A	A	A	A	A	A	A		A	A	A
11	A	A	A	A	A	A	G	R		R	G	R
12	•	•	•	•	•	•	•	•		•	•	•
13	A	A	A	A	A	A	A	A	A?	A	A	A
14	T	T	T	T	T	T	T	T	T?	T	T	T
15	G	G	G	G	G	G	G	G	G?	G	G	G
16	A	A	A	A	A	A	A	A		A	A	A
17	A	A	A	A	A	A	A	A		A	A	A
18	A	A	A	A	A	A	G	R		R	G	R
19	C	C	C	C	C	C	T	Y		Y	C	Y
20	T	T	T	T	T	T	T	T		T	T	T
21	C	C	C	C	C	C	G	C/G		C/G	C	C/G
22	C	C	C	C	C	C	C	C		C	C	C
23	T	T	T	T	T	T	T	T		T	T	T
24	C	C	C	C	C	C	C	C		C	C	C
25	T	T	T	T	T	T	T	T		T	C	Y
26	T	T	T	T	T	T	G	N		N	T	N
27	G	G	G	G	G	G	T	N		N	C	N
28	T	T	T	T	T	T	T	T		T	T	T
29	G	G	G	G	G	G	G	G		G	G	G
30	C	C	C	C	C	C	T	Y		Y	T	Y
31	T	T	T	T	T	T	C	Y	C	Y	C	Y
32	T	T	T	T	T	T	T	T	T	T	T	T
33	T	T	T	T	T	T	A	T/A	T	T/A	T	T/A
34	G	G	G	G	G	G	A	R	A	R	G	R
35	C	C	C	C	C	C	T	Y	C	Y	T	Y
36	G	T	T	T	G	N	C	N	T	N	C	N
37	T	T	T	T	T	T	T	T	T	T	C	Y
38	T	T	T	T	T	T	C	Y	C	Y	T	Y
39	C	C	C	C	C	C	C	C	T	Y	C	Y
40	G	G	G	G	G	G	C	G/C	C	G/C	G	G/C
41	C	C	C	C	C	C	T	Y	T	Y	T	Y
42	C	C	C	C	C	C	C	C	C	C	T	Y
43	G	G	G	G	G	G	A	R	A	R	G	R
44	C	C	C	C	C	C	T	Y	T	Y	C	Y
45	C	C	C	C	C	C	T	Y	T	Y	C	Y
46	A	A	A	A	A	A	C	N	C	N	T	N
47	T	T	T	T	T	T	T	T	T	T	T	T
48	C	C	C	C	C	C	G	C/G	G	C/G	G	C/G
49	G	G	G	G	G	G	T	N	T	N	G	N
50	T	T	T	T	T	T	T	T	T	T	T	T
51	C	C	C	C	C	C	G	C/G	G	C/G	C	C/G
52	A	A	A	A	A	A	G	R	G	R	G	R
53	T	T	T	T	T	T	T	T	T	T	C	Y
54	C	C	C	C	C	C	C	C	C	C	C	C
55	G	G	G	G	G	G	A	R	C	N	G	N
56	G	G	G	G	G	G	C	G/C	C	G/C	C	G/C
57	A	A	A	A	A	A	A	A	A	A	G	R
58	G	G	G	G	G	G	G	G	G	G	A	R
59	C	C	C	C	C	C	T	Y	T	Y	C	Y
60	T	T	T	T	T	T	T	T	T	T	C	Y
61	C	C	C	C	C	C	C	C	C	C	T	Y
62	A	A	A	A	A	A	A	A	A	A	A	A
63	G	G	G	G	G	G	G	G	G	G	C	N
64	G	G	G	G	G	G	G	G	G	G	G	G
65	C	C	C	C	C	C	C	C	C	C	C	C
66	T	T	T	T	T	T	C	Y	C	Y	C	Y
67	C	C	C	C	C	C	C	C	A	N		N
68	T	T	T	T	T	T	T	T	T	T		T
69	C	C	C	C	C	C	G	C/G	A	N		N
70	A	A	A	A	A	A	A	A	A	A		A
71	C	C	C	C	C	C	C	C	C	C		C
72	C	C	C	C	C	C	C	C	T	Y		Y
73	G	G	G	G	G	G	G	G	C	G/C	G	G/C
74	A	A	A	A	A	A	A	A	A	A	A	A
75	C	C	C	T	C	Y	G	N	G	N	A	N
76	G	G	G	G	G	G	G	G	G	G	A	R
77	A	A	A	A	A	A	C	N	A	N	C	N
78	A	A	A	A	A	A	A	A	A	A	A	A
79	C	C	C	C	C	C	C	C	G	C/G	C	C/G
80	A	A	A	A	A	A	A	A	A	A	C	N
81	G	G	G	G	G	G	A	R	C	N	T	N
82	A	A	A	A	A	A	A	A	C	N	C	N
83	T	T	T	T	T	T	T	T	T	T	G	N
84	A	A	A	A	A	A	T	A/T	T	A/T	T	A/T
85	C	C	C	C	C	C	G	C/G	G	C/G	G	C/G
86	A	A	A	A	A	A	A	A	A	A	A	A
87	G	G	G	G	G	G	G	G	G	G	G	G
88	A	A	A	A	A	A	A	A	C	N	A	N
89	A	A	A	A	A	A	A	A	T	A/T	A	A/T
90	A	A	A	A	A	A	A	A	A	A	G	R
91	A	A	A	A	A	A	C	N	C	N	C	N
92	G	G	G	G	G	G	T	N	T	N	T	N
93	G	G	G	G	G	G	G	G	G	G	G	G
94	A	A	A	A	A	A	A	A	C	N	A	N
95	A	A	A	A	A	A	A	A	C	N	A	N
96	C	C	C	C	C	C	C	C	C	C	G	Y
97	A	A	A	A	A	A	A	A	C	N	C	N
98	A	A	A	A	A	A	A	A	A	A	A	A
99	G	G	G	G	G	G	G	G	A	R	G	R
100	A	A	A	A	A	A	A	A	A	A	C	N
101	T	T	T	T	T	T	T	T	C	Y	A	N
102	C	C	C	C	C	C	C	C	C	C	C	C
103	A	A	A	A	A	A	A	A	A	A	A	A
104	G	G	G	G	G	G	G	G	G	G	G	G
105	C	C	C	C	C	C	C	C	C	C	C	C
106	A	A	A	A	A	A	A	A	G	R	G	R
107	A	A	A	A	A	A	A	A	C	N	A	N
108	A	A	A	A	A	A	A	A	A	A	C	N
109	G	G	G	G	G	G	A	R	G	R	G	R
110	A	A	A	A	A	A	A	A	A	A	C	N
111	A	A	A	A	G	R	A	R	G	R	C	N
112	T	T	T	T	T	T	T	T	T	T	T	T
113	G	G	G	G	G	G	G	G	G	G	G	G
114	C	C	C	C	C	C	T	Y	C	Y	C	Y
115	C	C	C	C	C	C	C	C	A	N	A	N
116	A	A	A	A	A	A	A	A	A	A	A	A
117	G	G	G	G	G	G	A	R	G	R	G	R

FIG. 7.2

Position	Tm 12.84-2 2	Tm 12 84-2.3	Tm 12 84-3 4	Tm 12 84-3 9	Tm 12 84-7 5	Concensus of	Tm13 17	Concensus with	B1	Concensus with	AFP-3	Concensus with
118	C	C	C	C	C	C	A	N	A	N	G	N
119	A	A	A	A	A	A	A	A	C	N	C	N
120	G	G	G	G	G	G	T	N	T	N	T	N
121	G	G	G	G	G	G	G	G	G	G	G	G
122	T	T	T	A	T	T/A	A	T/A	A	T/A	A	A/T
123	G	G	G	G	G	G	A	R	A	R	G	R
124	T	T	T	T	T	T	A	T/A	A	T/A	T	T/A
125	C	C	C	C	C	C	G	C/G	G	C/G	C	C/G
126	C	C	C	C	C	C	T	Y	T	Y	T	Y
127	G	G	G	G	G	G	G	G	G	G	G	G
128	G	G	G	G	G	G	G	G	G	G	G	G
129	A	A	A	A	A	A	A	A	A	A	A	A
130	G	G	G	G	G	G	G	G	G	G	G	G
131	T	T	T	T	T	T	T	T	T	T	T	T
132	G	G	G	G	G	G	G	G	G	G	A	R
133	T	T	T	T	T	T	T	T	T	T	T	T
134	C	C	C	C	C	C	C	C	C	C	C	C
135	C	C	C	C	C	C	G	C/G	C	C/G	T	N
136	C	C	C	C	C	C	C	C	G	C/G	G	C/G
137	A	A	A	A	A	A	A	A	A	A	A	A
138	A	A	A	A	A	A	A	A	A	A	A	A
139	G	G	G	G	G	G	G	G	G	G	G	G
140	A	A	A	A	A	A	A	A	A	A	A	A
141	G	G	G	G	G	G	G	G	C	G/C	G	G/C
142	A	A	A	A	A	A	A	A	G	R	T	N
143	C	C	C	C	C	C	T	Y	T	Y	C	Y
144	G	G	G	G	G	G	C	G/C	C	G/C	C	G/C
145	A	A	A	A	A	A	A	A	A	A	C	N
146	T	T	T	T	T	T	T	T	T	T	T	T
147	C	C	C	C	C	C	A	N	A	N	C	N
148	G	G	G	G	G	G	A	R	A	R	A	R
149	A	A	A	A	A	A	C	N	A	N	A	N
150	C	C	C	C	C	C	C	C	G	C/G	C	C/G
151	A	A	A	A	A	A	A	A	A	A	A	A
152	A	A	A	A	A	A	A	A	G	R	A	R
153	A	A	A	A	A	A	A	A	A	A	G	R
154	G	G	G	G	G	G	G	G	G	G	G	G
155	T	T	T	T	T	T	C	Y	C	Y	T	Y
156	C	C	C	C	C	C	T	Y	T	Y	T	Y
157	C	C	C	C	C	C	C	C	C	C	C	C
158	G	G	G	G	G	G	G	G	G	G	G	G
159	C	C	C	C	C	C	C	C	C	C	C	C
160	A	A	A	A	A	A	A	A	A	A	A	A
161	C	C	C	C	C	C	A	N	A	N	A	N
162	A	A	A	A	A	A	C	N	A	N	C	N
163	G	G	G	G	G	G	G	G	G	G	C	G/C
164	G	G	G	G	G	G	G	G	G	G	G	G
165	T	T	T	T	T	T	T	T	T	T	T	T
166	G	G	G	G	G	G	G	G	G	G	G	G
167	T	T	T	T	T	T	A	T/A	A	T/A	A	T/A
168	C	C	C	C	C	C	C	C	C	C	A	N
169	T	T	T	T	T	T	T	T	T	T	G	N
170	T	T	T	T	T	T	G	N	T	N	A	N
171	G	G	G	G	G	G	G	G	G	G	A	R
172	G	G	G	G	G	G	G	G	G	G	G	G
173	T	T	T	T	T	T	A	T/A	A	T/A	T	T/A
174	C	C	C	C	C	C	G	C/G	G	C/G	G	C/G
175	G	G	G	G	G	G	G	G	G	G	G	G
176	A	A	A	A	A	A	A	A	A	A	A	A
177	T	C	C	C	C	Y	C	Y	C	Y	C	Y
178	G	G	G	G	G	G	G	G	G	G	G	G
179	A	A	A	A	A	A	A	A	A	A	A	A
180	T	T	T	T	T	T	T	T	C	Y	C	Y
181	C	C	C	C	C	C	C	C	C	C	C	C
182	C	C	C	C	C	C	C	C	C	C	C	C
183	C	C	C	C	C	C	T	Y	C	Y	C	Y
184	A	A	A	A	A	A	A	A	A	A	A	A
185	A	A	A	A	A	A	A	A	A	A	A	A
186	A	A	A	A	A	A	A	A	A	A	A	A
187	A	A	A	A	A	A	C	N	C	N	C	N
188	T	T	T	T	T	T	T	T	T	T	T	T
189	G	G	G	G	G	G	G	G	G	G	G	G
190	A	A	A	A	A	A	A	A	A	A	A	A
191	A	A	A	A	A	A	A	A	A	A	A	A
192	G	G	G	G	G	G	A	R	A	R	A	R
193	A	A	A	A	A	A	C	N	A	N	G	N
194	A	A	A	A	A	A	G	R	T	N	A	N
195	G	G	G	G	G	G	C	G/C	G	G/C	G	G/C
196	C	C	C	C	C	C	C	C	C	C	C	C
197	A	A	A	A	A	A	A	A	A	A	A	A
198	C	C	C	C	C	C	A	N	A	N	T	N
199	G	G	G	G	G	G	G	G	C	G/C	G	G/C
200	T	T	T	T	T	T	T	T	T	T	C	Y
201	C	C	C	C	C	C	T	Y	T	Y	T	Y
202	C	C	C	C	C	C	T	Y	C	Y	T	Y
203	T	T	T	T	T	T	T	T	T	T	T	T
204	C	C	C	C	C	C	T	Y	T	Y	C	Y
205	T	T	T	T	T	T	T	T	T	T	T	T
206	G	G	G	G	G	G	G	G	G	G	G	G
207	C	C	C	C	C	C	C	C	C	C	C	C
208	T	T	T	T	T	T	G	N	A	N	A	N
209	T	T	T	T	T	T	T	T	T	T	T	T
210	C	C	C	C	C	C	G	C/G	T	N	C	N
211	T	T	T	T	T	T	G	N	T	N	T	N
212	C	C	C	C	C	C	C	C	T	Y	T	Y
213	G	G	G	G	G	G	C	G/C	C	G/C	G	G/C
214	A	A	A	A	A	A	A	A	A	A	A	A
215	A	A	A	A	A	A	G	R	A	R	A	R
216	G	G	G	G	G	G	G	G	G	G	G	G
217	A	A	A	A	A	A	A	A	G	R	A	R
218	A	A	A	G	A	R	A	R	C	N	G	N
219	A	A	A	A	A	A	C	N	A	N	A	N
220	A	A	A	A	A	A	G	R	C	N	G	N
221	C	C	C	C	C	C	C	C	T	Y	C	Y
222	T	T	T	T	T	T	C	Y	C	Y	C	Y
223	G	G	G	G	G	G	G	G	G	G	G	G
224	G	G	G	G	G	G	G	G	A	R	G	R
225	A	A	A	A	A	A	T	A/T	A	A/T	A	A/T
226	G	G	G	G	G	G	C	G/C	A	N	T	N
227	T	T	T	T	T	T	T	T	T	T	T	T
228	G	G	G	G	G	G	G	G	A	R	C	N
229	G	G	G	G	G	G	G	G	G	G	A	R
230	C	C	C	C	C	C	C	C	T	Y	T	Y
231	A	A	A	A	A	A	C	N	C	N	C	N
232	A	A	A	A	A	A	A	A	G	R	G	R
233	C	C	C	C	C	C	C	C	C	C	A	N
234	C	C	C	C	C	C	G	C/G	G	C/G	C	C/G
235	G	G	G	G	G	G	G	G	G	G	G	G

FIG. 7.2 Cont.

Position	Tm 12.84-2 2	Tm 12.84-2 3	Tm 12.84-3 4	Tm 12.84-3 9	Tm 12.84-7 5	Concensus of	Tm13 17	Concensus with	B1	Concensus with	AFP 3	Concensus with
236	A	A	A	A	A	A	A	A	A	A	C	N
237	A	A	A	A	A	A	A	A	A	A	C	N
238	G	G	G	G	G	G	T	N	T	N	A	N
239	C	C	C	C	C	C	C	C	C	C	G	C/G
240	C	C	C	C	C	C	G	C/G	G	C/G	T	N
241	G	G	G	G	G	G	G	G	G	G	G	G
242	G	G	G	G	G	G	G	G	G	G	G	G
243	A	A	A	A	A	A	A	A	A	A	T	A/T
244	G	G	G	G	G	G	G	G	G	G	G	G
245	A	A	A	A	A	A	A	A	A	A	A	A
246	C	C	C	C	C	C	G	C/G	A	N	A	N
247	A	A	A	A	A	A	G	R	A	R	T	N
248	C	C	C	C	C	C	T	Y	T	Y	T	Y
249	C	C	C	C	C	C	G	C/G	T	N	C	N
250	A	A	A	A	A	A	G	R	G	R	C	N
251	A	A	A	A	A	A	T	A/T	A	A/T	A	A/T
252	T	T	T	T	T	T	G	N	G	N	A	N
253	G	G	G	G	G	G	G	G	G	G	C	G/C
254	T	T	T	T	T	T	T	T	C	Y	T	Y
255	G	G	G	G	G	G	C	G/C	C	G/C	G	G/C
256	G	G	G	G	G	G	G	G	G	G	G	G
257	A	A	A	A	A	A	A	A	A	A	A	A
258	G	G	G	G	G	G	C	G/C	C	G/C	C	G/C
259	G	G	G	G	G	G	G	G	A	R	C	N
260	T	T	T	T	T	T	T	T	C	Y	A	N
261	A	A	A	A	A	A	G	R	G	R	C	N
262	C	C	C	C	C	C	T	Y	T	Y	A	N
263	T	T	T	T	T	T	T	T	T	T	T	T
264	C	C	C	C	C	C	G	C/G	C	C/G	T	N
265	A	A	A	A	A	A	A	A	A	A	A	A
266	A	A	A	A	A	A	G	R	A	R	A	R
267	A	A	A	A	A	A	G	R	G	R	G	R
268	G	G	G	G	G	G	G	G	G	G	A	R
269	C	C	C	C	C	C	A	N	A	N	C	N
270	C	C	C	C	C	C	G	C/G	G	C/G	G	C/G
271	A	A	A	A	A	A	A	A	A	A	A	A
272	A	A	A	A	A	A	A	A	A	A	A	A
273	G	G	G	G	G	G	G	G	G	G	A	R
274	C	C	C	C	C	C	G	C/G	T	N	T	N
275	T	T	T	T	T	T	T	T	T	T	T	T
276	G	G	G	G	G	G	G	G	G	G	C	G/C
277	A	A	A	A	A	A	A	A	A	A	A	A
278	A	A	A	A	A	A	G	R	C	N	A	N
279	G	G	G	G	G	G	G	G	G	G	G	G
280	C	C	C	C	C	C	A	N	A	N	G	N
281	A	A	A	A	A	A	A	A	G	R	A	R
282	T	T	T	T	T	T	G	N	G	N	A	N
283	G	G	G	G	G	G	G	G	T	N	A	N
284	T	T	T	T	T	T	T	T	G	N	A	N
285	G	G	G	G	G	G	C	G/C	A	N	C	N
286	G	G	G	G	G	G	A	R	C	N	T	N
287	C	C	C	C	C	C	C	C	A	N	C	N
288	C	C	C	C	C	C	T	Y	A	N	T	N
289	A	A	A	A	A	A	G	R	A	R	G	R
290	G	G	G	G	G	G	A	R	C	N	A	N
291	C	C	C	C	C	C	C	C	G	C/G	G	C/G
292							A	A	A	A	G	R
293							A	A	T	A/T	A	A/T
294							C	C	G	C/G	T	N
295	G	G	G	G	G	G	G	G	A	R	T	N
296	A	A	A	A	A	A	A	A	T	A/T	C	N
297	C	C	C	C	C	C	C	C	G	C/G	G	C/G
298	G	G	G	G	G	G	G	G	A	R	G	R
299	A	A	A	A	A	A	A	A	A	A	A	A
300	A	A	A	A	A	A	A	A	G	R	G	R
301	G	G	G	G	G	G	G	G	A	R	A	R
302	A	A	A	A	A	A	A	A	A	A	A	A
303	G	A	G	A	G	R	A	R	A	R	G	R
304	G	G	G	G	G	G	A	R	G	R	G	R
305	T	T	T	T	T	T	C	Y	C	Y	T	Y
306	G	G	G	G	G	G	T	N	G	N	C	N
307	G	G	G	G	G	G	G	G	A	R	G	R
308	A	A	A	A	A	A	A	A	G	R	A	R
309	C	C	C	C	C	C	G	C/G	A	N	T	N
310	A	A	A	A	A	A	A	A	A	A	G	R
311	A	A	A	A	A	A	A	A	G	R	A	R
312	G	G	G	G	G	G	A	R	A	R	C	N
313	A	A	A	A	A	A	A	A	T	A/T	T	A/T
314	T	T	T	T	T	T	T	T	T	T	T	T
315	C	C	C	C	C	C	C	C	G	C/G	G	C/G
316	G	G	G	G	G	G	A	R	T	N	G	N
317	T	T	T	T	T	T	T	T	C	Y	T	Y
318	G	G	G	G	G	G	C	G/C	G	G/C	T	N
319	C	C	C	C	C	C	A	N	A	N	G	N
320	A	A	A	A	A	A	A	A	G	R	C	N
321	G	G	G	G	G	G	T	N	A	N	C	N
322	A	A	A	A	A	A	A	A	A	A	A	A
323	A	A	A	A	A	A	A	A	G	R	A	R
324	G	G	G	G	G	G	G	G	T	N	A	N
325	T	T	T	T	T	T	T	T	G	N	T	N
326	G	G	G	G	G	G	G	G	C	G/C	G	G/C
327	C	C	C	C	C	C	C	C	A	N	T	N
328	G	G	G	G	G	G	G	G	C	G/C	G	G/C
329	T	T	T	T	T	T	C	Y	G	N	C	N
330	G	G	G	G	G	G	C	G/C	G	G/C	C	G/C
331	G	G	G	G	G	G	G	G	T	N	G	N
332	T	T	T	T	T	T	T	T	G	N	T	N
333	C	C	C	C	C	C	C	C	A	N	C	N
334	A	A	A	A	A	A	A	A	C	N	A	N
335	A	A	A	A	A	A	A	A	T	N	A	N
336	G	G	G	G	G	G	G	G	G	G	G	G
337	A	A	A	A	A	A	A	A	A	A	A	A
338	A	A	A	A	A	A	G	R	A	R	A	R
339	G	G	G	G	G	G	A	R	C	N	G	N
340	G	G	G	G	G	G	G	G	A	R	G	R
341	C	C	C	C	C	C	A	N	C	N	A	N
342	C	C	C	C	C	C	T	Y	A	N	C	N
343	A	A	A	A	A	A	A	A	C	N	A	N
344	C	C	C	C	C	C	C	C	T	Y	C	Y
345	A	A	A	A	A	A	T	A/T	C	N	T	N
346	C	C	C	C	C	C	G	C/G	C	C/G	C	C/G
347	C	C	C	C	C	C	T	Y	G	N	C	N
348	A	A	A	A	A	A	T	A/T	G	N	T	N
349	G	G	G	G	G	G	G	G	A	R	C	N
350	A	A	A	A	A	A	A	A	A	A	A	A
351	G	G	G	G	G	G	A	R	G	R	G	R
352	G	G	G	G	G	G	G	G	A	R	C	N
353	A	A	A	A	A	A	A	A	T	A/T	A	A/T

FIG. 7.2 Cont.

Position	Tm 12.84-2 2	Tm 12.84-2 3	Tm 12.84-3.4	Tm 12.84-3 9	Tm 12.84-7.5	Concensus of	Tm13 17	Concensus with	B1	Concensus with	AFP-3	Concensus with
354	A	A	A	A	A	A	G	R	A	R	C	N
355	A	A	A	A	A	A	A	A	C	N	T	N
356	C	C	C	C	C	C	C	C	G	C/G	C	C/G
357	G	G	G	G	G	G	G	G	C	G/C	C	G/C
358	G	G	G	G	G	G	G	G	C	G/C	T	N
359	C	C	C	C	C	C	T	Y	A	N	C	N
360	T	T	T	T	T	T	G	N	T	N	A	N
361	T	T	T	T	T	T	T	T	T	T	G	N
362	A	A	A	A	A	A	T	A/T	T	A/T	C	N
363	T	T	T	T	T	T	C	Y	G	N	C	N
364	G	G	G	G	G	G	A	R	A	R	G	R
365	A	A	A	A	A	A	A	A	A	A	A	A
366	C	C	C	C	C	C	T	Y	G	N	C	N
367	A	A	A	A	A	A	A	A	T	A/T	T	A/T
368	C	C	C	C	C	C	C	C	T	Y	T	Y
369	C	C	C	C	C	C	T	Y	A	N	C	N
370	T	T	T	T	T	T	T	T	C	Y	T	Y
371	T	T	T	T	T	T	T	T	C	Y	T	Y
372	C	C	C	C	C	C	C	C	A	N	T	N
373	A	A	A	A	A	A	A	A	A	A	A	A
374	A	A	A	A	A	A	A	A	A	A	A	A
375	G	G	G	G	G	G	A	R	T	N	A	N
376	T	T	G	T	T	N	T	N	G	N	T	N
377	G	G	T	G	G	N	G	N	T	N	G	N
378	T	T	T	T	T	T	T	T	G	N	C	N
379	A	A	A	A	A	A	G	R	T	N	G	N
380	T	T	T	T	T	T	T	T	A	T/A	T	T/A
381	T	T	T	T	T	T	C	Y	T	Y	T	Y
382	T	T	T	T	T	T	A	T/A	T	T/A	C	N
383	A	A	A	A	A	A	T	A/T	G	N	A	N
384	C	C	C	C	C	C	G	C/G	A	N	C	N
385	G	G	G	G	G	G	A	R	A	R	G	R
386	A	A	A	A	A	A	A	A	G	R	A	R
387	C	C	C	C	C	C	A	N	G	N	T	N
388	A	A	A	A	A	A	A	A	A	A	A	A
389	G	G	G	G	G	G	A	R	C	N	A	N
390	C	T	T	T	T	T	C	Y	A	N	C	N
391	A	A	A	A	A	A	A	A	A	A	A	A
392	A	A	A	A	A	A	A	A	G	R	G	R
393	A	A	A	A	A	A	G	R	C	N	G	N
394	C	C	C	C	C	C	C	C	C	C	T	Y
395	C	C	C	C	C	C	C	C	C	C	C	C
396	T	T	T	T	T	T	A	T/A	A	T/A	G	N
397												
398	G	G	G	G	G	G	A	R	A	R		
399	A	A	A	A	A	A	A	A	T	A/T		
400	T	T	T	T	T	T	G	N	T	N		
401	T	T	T	T	T	T	T	T	T	T		
402	T	T	T	T	T	T	T	T	C	Y		
403	C	C	C	C	C	C	C	C	T	Y		
404	T	T	T	T	T	T	T	T	T	T		
405	C	C	C	C	C	C	C	C	T	Y		
406	T	T	T	T	T	T	A	T/A	G	N		
407	C	C	C	C	C	C	C	C	C	C		
408	C	C	C	C	C	C	C	C	A	N		
409	T	T	T	T	T	T	A	T/A	G	N		
410	A	A	A	A	A	A	G	R	A	R		
411	T	T	T	T	T	T	T	T	C	Y		
412	T	T	T	T	T	T	T	T	T	T		
413	G	G	G	G	G	G	G	G	A	R		
414	A	A	A	A	A	A	A	A	T	A/T		
415	T	T	T	T	T	T	T	T	T	T		
416	•	•	•	•	•	•	•	•		•	•	•
417	T	T	T	T	T	T	T	T	T	T	T	T
418	A	A	A	A	A	A	G	R	G	R	A	R
419	A	A	A	A	A	A	A	A	T	A/T	A	A/T
420	T	T	T	T	T	T	A	T/A	C	N	T	N
421	T	T	T	T	T	T	C	Y	T	Y	G	N
422	G	G	G	G	G	G	C	G/C	G	G/C	G	N
423	T	T	T	T	T	T	A	T/A	A	T/A	G	N
424	T	T	T	T	T	T	C	Y	A	N	G	N
425	T	T	T	T	T	T	C	Y	A	N	T	N
426	T	T	T	T	T	T	A	T/A	G	N	C	N
427	G	G	G	G	G	G	C	G/C	C	G/C	G	N
428	T	T	T	T	T	T	G	N	T	N	C	N
429	A	A	A	A	A	A	A	A	T	A/T	T	N
430	T	T	T	T	T	T	C	Y	T	Y	C	Y
431	T	T	T	T	T	T	T	T	G	N	G	N
432	T	T	T	T	T	T	A	T/A	T	T/A	A	T/A
433	G	G	G	G	G	G	G	G	G	G	A	R
434	A	A	A	A	G	R	T	N	C	N	G	N
435	C	C	C	C	C	C	A	N	A	N	A	N
436	T	T	T	T	T	T	G	N	C	N	C	N
437	G	G	G	G	G	G	A	R	A	R	T	A/T
438	A	A	A	A	A	A	T	A/T	A	A/T	T	A/T
439	A	A	A	A	A	A	G	R		R	A	R
440	T	T	T	T	T	T	G	N		N	T	N
441	T	T	T	T	T	T	T	T		T	T	T/A
442	T	T	T	T	T	T	T	T		T	A	T/A
443	T	T	T	T	T	T	C	Y		Y	T	Y
444	G	G	G	G	G	G	A	R		R	A	R
445	A	A	A	A	A	A	A	A		A	A	A
446	C	C	C	C	C	C	A	N		N	T	N
447							T	T		T	G	N
448							G	G		G	T	N
449							G	G		G	A	R
450							T	T		T	A	T/A
451							G	G		G	A	R
452							T	T		T	C	Y
453							G	G		G	C	G/C
454							C	C		C	T	Y
455							T	T		T	T	T
456							T	T		T	G	N
457							T	T		T	C	Y
458							A	A		A	C	N
459							C	C		C	A	N
460							A	A		A	A	A
461							T	T		T	T	T
462							A	A		A	T	A/T
463							T	T		T	T	T
464							A	A		A	T	A/T
465							A	A		A	T	A/T
466							A	A		A	T	A/T
467											T	N
468											G	N
469											A	R
470											A	T/A
471											C	G/C

FIG. 7.2 Cont.

Position	Tm 12 84-2 2	Tm 12 84-2 3	Tm 12 84-3 4	Tm 12 84-3.9	Tm 12.84-7 5	Concensus of	Tm13.17	Concensus with	B1	Concensus with	AFP-3	Concensus with
472	A	A	A	A	A	A	A	A		A	A	A
473	A	A	A	A	A	A	A	A		A	A	A
474	T	T	T	T	T	T	T	T		T	T	T
475	A	A	A	A	A	A	A	A		A	A	A
476	A	A	A	A	A	A	A	A		A	A	A
477	A	A	A	A	A	A	A	A		A	A	A
478	G	G	G	G	G	G	G	G		G	T	N
479	G	G	G	G	G	G	T	N		N	T	N
480	T	T	T	T	T	T	G	N		N	T	N
481	A	A	A	A	A	A	T	N		N	A	N
482	A	C	C	C	C	C	T	Y		Y	A	N
483	T	T	T	T	T	T	T	T		T	A	A/T
484	A	A	A	A	A	A	C	N		N	G	N
485	T	T	T	T	T	T	T	T		T	A	A/T
486	C	C	C	C	C	C	G	N		N	A	N
487	G	G	G	G	G	G	A	R		R	A	R
488	T	T	T	T	T	T	T	T		T	A	T/A
489	T	T	T	T	T	T	G	N		N	A	N
490	A	A	A	A	A	A	T	N		N	A	N
491	T	T	T	T	T	T	A	N		N	A	R
492	G	G	G	G	G	G	A	R		R	A	N
493	T	A	T	A	T	N	A	N		N	A	N
494	A	A	A	A	A	A	A	A		A	A	A
495	A	A	A	A	A	A	A	A		A	A	A
496	A	A	A	A	A	A	A	A		A	A	A
497	A	A	A	A	A	A	A	A		A	A	A
498	A	A	A	A	A	A	A	A		A	A	A
499	A	A	A	A	A	A	A	A		A	A	A
500	A	A	A	A	A	A	A	A		A	A	A
501	A	A	A	A	A	A	A	A		A	A	A
502	A	A	A	A	A	A	A	A		A	A	A
503	A	A	A	A	A	A	A	A		A		A
504	A	A	A	A	A	A	A	A		A		A
505	A	A	A	A	A	A	A	A		A		A
506	A	A	A	A	A	A	A	A		A		A
507	A	A	A	A	A	A	A	A		A		A
508	A	A	A	A	A	A	A	A		A		A
509	A	A	A	A	A	A	A	A		A		A
510	A	A	A	A	A	A	A	A		A		A
511	A	A	A	A	A	A	A	A		A		A
512												

FIG. 7.2 Cont.

Position	Tm 12.84-2.2	Tm 12.84-2.3	Tm 12.84-3.4	Tm 12.84-3.9	Tm 12.84-7.5	Concensus Tm 12.84	Tm 13.17	Concensus to Tm13.17	Tm P-B1	Tm P-B2	Concensus to B1/B2	Tm AFP-3	Concensus to AFP-3	GENERAL CONCENSUS	SUBSTITUTIONS - most to least common			
1	M	M	M	M	M	M	M	M			M, OR GAP	M	M, OR GAP	M				
2	K	K	K	K	K	K	K	K			K, OR GAP	K	K, OR GAP	K				
3	L	L	L	L	L	L	L	L			L, OR GAP	L	L, OR GAP	L				
4	L	L	L	L	L	L	L	L			L, OR GAP	L	L, OR GAP	L				
5	L	L	L	L	L	L	C	HY-, ALP/SUL			HY-, ALP/SUL, OR GAP	L	HY-, ALP/SUL, OR GAP	L	C			
6	C	C	C	C	C	C	C	C			C OR GAP	C	C OR GAP	C				
7	F	F	F	F	F	F	L	HY- ARO/ALP	L	L	HY- ARO/ALP	L	HY-, ARO/ALP	F	L			
8	A	A	A	A	A	A	I	ALP	T	T	ALP/ALH	V	ALP/ALH	A	I	T	V	
9	F	F	F	F	F	F	S	ARO/ALH	S	S	ARO/ALH	L	ARO/ALH/ALP	F	S	L		
10	A	A	A	A	A	A	L	ALP	L	L	ALP	V	ALP	A	L	V		
11	A	A	A	A	A	A	I	ALP	I	I	ALP	A	ALP	A	I			
12	I	I	I	I	I	I	L	ALP	L	L	ALP	L	ALP	I	L			
13	V	V	V	V	V	V	L	ALP	L	L	ALP	V	ALP	V	L			
14	I	I	I	I	I	I	V	ALP	V	V	ALP	A	ALP	I	V	A		
15	G	G	G	G	G	G	T	ALP/ALH	A	A	ALP/ALH	A	ALP/ALH	G	T	A		
16	A	A	A	A	A	A	V	ALP	V	V	ALP	T	ALP/ALH	A	V	T		
17	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Y	ACD/ARO	Q	Y			
18	A	A	A	A	A	A	A	A	A	A	A	A	A	A				
19	L	L	L	L	L	L	L	L	I	L	ALP		ALP OR GAP	L	I			
20	T	T	T	T	T	T	T	T	T	T	T		T OR GAP	T				
21	D	D	D	D	D	D	E	ACD	E	E	ACD	E	ACD	D	E			
22	E	E	E	E	E	E	A	ACD/ALP	E	E	ACD/ALP	T	ACD/ALP/ALH	E	A	T		
23	Q	Q	Q	Q	Q	Q	Q	Q	D	D	ACD	P	ACD/ALP	Q	D	P		
24	I	I	I	I	I	I	I	I	L	L	ALP	R	ALP/BAS	I	L	R		
25	Q	Q	Q	Q	Q	Q	E	GLX	E	Q	GLX	E	GLX	Q	E			
26	K	K	K	K	K	K	K	K	L	L	BAS/ALP	K	BAS/ALP	K	L			
27	R	R	R	R	R	R	L	BAS/ALP	L	L	BAS/ALP	L	BAS/ALP	R	L			
28	N	N	N	N	N	N	N	N	R	R	HY+ BAS/ACD	K	HY+ BAS/ACD	N	R	K		
29	K	K	K	K	K	K	K	K	Q	Q	HY+, BAS/ACD	Q	HY+ BAS/ACD	K	Q			
30	I	I	I	I	I	I	I	I	T	T	ALP/ALH	H	ALP/ALH/BAS	I	T	H		
31	S	S	S	S	S	S	S	S	S	S	S	S	S	S				
32	K	K	K	K	K	K	K	K	A	A	BAS/ALP	D	BAS/ALP/ACD	K	A	D		
33	E	E	E	E	E	E	K	HY+ ACD/BAS	E	E	HY+, ACD/BAS	A	ACD/BAS/ALP	E	K	A		
34	C	C	C	C	C	C	C	C	C	C	C	C	C	C				
35	Q	Q	Q	Q	Q	Q	Q	Q	K	K	HY+ ACD/BAS	K	HY+ ACD/BAS	Q	K			
36	Q	Q	Q	Q	Q	Q	N	ACD	T	T	HY+, ACD/ALH	A	ACD/ALH/ALP	Q	N	T	A	
37	V	V	V	E	V	ALP/ACD	E	ACD/ALP	E	E	ACD/ALP	E	ACD/ALP	E	V		(3 9, Tm13 17 B1, B2, AFP-3)	
38	S	S	S	S	S	S	S	S	S	S	S	S	S	S				
39	G	G	G	G	G	G	G	G	G	G	G	G	G	G				
40	V	V	V	V	V	V	V	V	V	A	ALP	V	ALP	V	A			
41	S	S	S	S	S	S	S	S	S	S	S	S	S	S				
42	Q	Q	Q	Q	Q	Q	Q	Q	E	E	GLX	E	GLX	Q	E			
43	E	E	E	E	E	E	E	E	D	A	ACD/ALP	E	ACD/ALP	E	D	A		
44	T	T	T	T	T	T	I	ALP	V	V	ALP	S	ALP/ALH	T	I	V	S	
45	I	I	I	I	I	I	I	I	I	I	I	L	ALP	I	L			
46	D	D	D	D	D	D	T	HY+ ACD/ALH	K	K	HY+, ACD/ALH/BAS	N	HY+ ACD/ALH/BAS	D	T	K	N	
47	K	K	K	K	K	K	K	K	R	K	BAS	K	BAS	K	R			
48	V	V	V	V	V	V	A	ALP	A	A	ALP	V	ALP	V	A			
49	R	R	R	R	R	R	R	R	R	R	R	R	R	R				
50	T	T	T	T	T	T	N	HY+ ALH/ACD	K	K	HY+, ALH/ACD/BAS	N	HY+, ALH/ACD/BAS	T	N	K		
51	G	G	G	G	G	G	G	G	G	G	G	R	ALP/BAS	G	R			
52	V	V	V	V	V	V	D	ALP/ACD	D	D	ALP/ACD	E	ALP/ACD	V	D	E		
53	L	L	L	L	L	L	W	HY- ALP/ARO	L	L	HY- ALP/ARO	E	ALP/ARO/ACD	L	W	E		
54	V	V	V	V	V	V	E	ALP/ACD	E	E	ALP/ACD	V	ALP/ACD	V	E			
55	D	D	D	D	D	D	D	D	D	D	D	D	D	D				
56	D	D	D	D	D	D	D	D	D	D	D	D	D	D				
57	P	P	P	P	P	P	P	P	P	P	P	P	P	P				
58	K	K	K	K	K	K	K	K	K	K	K	K	K	K				
59	M	M	M	M	M	M	L	HY-, SUL/ALP	L	L	HY- SUL/ALP	L	HY- SUL/ALP	M	L			
60	K	K	K	K	K	K	K	K	K	K	K	K	K	K				
61	K	K	K	K	K	K	R	BAS	M	M	BAS/SUL	E	BAS/SUL/ACD	K	R	M	E	
62	H	H	H	H	H	H	Q	HY+, BAS/ACD	Q	Q	HY+, BAS/ACD	H	HY+ BAS/ACD	H	Q			
63	V	V	V	V	V	V	V	V	L	L	ALP	A	ALP	V	L	A		
64	L	L	L	L	L	L	F	HY- ALP/ARO	L	L	HY- ALP/ARO	F	HY- ALP/ARO	L	F			
65	C	C	C	C	C	C	C	C	C	C	C	C	C	C				
66	F	F	F	F	F	F	V	HY-, ARO/ALP	I	I	HY- ARO/ALP	I	HY-, ARO/ALP	F	V	I		
67	S	S	S	S	S	S	A	ALH/ALP	F	F	ALH/ALP/ARO	L	ALH/ALP/ARO	S	A	F	L	
68	K	K	K	K	K	K	R	BAS	K	K	BAS	K	BAS	K	R			
69	K	K	K	R	K	BAS	N	HY+, BAS/ACD	A	A	BAS/ACD/ALP	R	BAS/ACD/ALP	K	R	A	N	(22 23 34 75)
70	T	T	T	T	T	T	A	ALP	L	L	ALP	A	ALP	T	A	L		
71	G	G	G	G	G	G	G	G	I	E	ALP/ACD	G	ALP/ACD	G	I	E		
72	V	V	V	V	V	V	L	ALP	E	I	ALP/ACD	F	ALP/ACD/ARO	V	L	I	E	F
73	A	A	A	A	A	A	A	A	V	V	ALP	I	ALP	A	V	I		
74	T	T	T	T	T	T	T	T	A	A	ALH/ALP	D	ALH/ALP/ACD	T	A	D		
75	E	E	E	E	E	E	E	E	E	E	E	A	ACD/ALP	E	A			
76	A	A	A	A	A	A	S	ALP/ALH	S	S	ALP/ALH	S	ALP/ALH	S	A			
77	G	G	G	G	G	G	G	G	G	G	G	G	G	G				
78	D	D	D	D	D	D	E	ACD	E	E	ACD	E	ACD	E	D			
79	T	T	T	T	T	T	V	ALP	I	I	ALP	F	ALP/ARO	T	I	V	F	
80	N	N	N	N	N	N	V	ACD/ALP	E	E	ACD/ALP	Q	ACD/ALP	N	E	V	Q	
81	V	V	V	V	V	V	V	V	A	A	ALP	L	ALP	V	A	L		
82	E	E	E	E	E	E	D	ACD	D	D	ACD	D	ACD	D	E			
83	V	V	V	V	V	V	V	V	T	T	ALP/ALH	H	ALP/ALH/BAS	V	T	H		
84	L	L	L	L	L	L	L	L	F	F	ALP/ARO	I	ALP/ARO	L	F	I		
85	K	K	K	K	K	K	R	BAS	K	K	BAS	K	BAS	K	R			
86	A	A	A	A	A	A	E	ALP/ACD	E	E	ALP/ACD	T	ALP/ACD/ALH	A	E	T		
87	K	K	K	K	K	K	K	K	K	K	K	K	K	K				
88	L	L	L	L	L	L	V	ALP	L	L	ALP	F	ALP/ARO	L	V	F		
89	K	K	K	K	K	K	R	BAS	T	T	BAS/ALH	K	BAS/ALH	K	T	R		
90	H	H	H	H	H	H	K	BAS	R	R	BAS	E	BAS/ACD	H	R	K	E	
91	V	V	V	V	V	V	K	V	V	V	V	N	ALP/ACD	V	N			
92	A	A	A	A	A	A	T	ALP/ALH	T	T	ALP/ALH	S	ALP/ALH	A	T	S		
93	S	S	S	S	S	S	D	HY+ ALH/ACD	N	N	HY+ ALH/ACD	E	HY+ ALH/ACD	S	N	D	E	
94							N	N OR GAP	D	D	ACD OR GAP	H	ACD OR GAP	D	N	H		
95	D	D	D	D	D	D	D	D	D	D	D	P	ACD/ALP	D	P			
96	E	E	E	E	E	E	E	E	E	E	E	L	E	E				
97	E	E	E	E	E	E	E	E	E	E	E	K	HY+ ACD/BAS	E	K			

FIG. 7.3

Position	Tm 12.84-2.2	Tm 12.84-2.3	Tm 12.84-3.4	Tm 12.84-3.9	Tm 12.84-7.5	Concensus	Tm 13.17	Concensus	Tm P-B1	Tm P-B2	Concensus	Tm AFP-3	Concensus	GENERAL	SUBSTITUTIONS - most to least common		
98	V	V	V	V	V	V	T	ALP/ALH	S	S	ALP/ALH	V	ALP/ALH	V	S	T	
99	D	D	D	D	D	D	E	ACD	E	E	ACD	D	ACD	D	E		
100	K	K	K	K	K	K	K	K	K	K	K	D	HY+, BAS/ACD	K	D		
101	I	I	I	I	I	I	I	I	I	I	I	L	ALP	I	L		
102	V	V	V	V	V	V	I	ALP	V	V	ALP	V	ALP	V	I		
103	Q	Q	Q	Q	Q	Q	N	ACD	E	E	ACD	A	ACD/ALP	Q	E	N	A
104	K	K	K	K	K	K	K	K	K	K	K	K	K	K			
105	C	C	C	C	C	C	C	C	C	C	C	C	C	C			
106	V	V	V	V	V	V	A	ALP	T	T	ALP/ALH	A	ALP/ALH	V	A	T	
107	V	V	V	V	V	V	V	V	V	V	V	V	V	V			
108	K	K	K	K	K	K	K	K	T	T	HY+ ACD/ALH	K	HY+, ACD/ALH	K	T		
109	K	K	K	K	K	K	R	BAS	E	E	HY+, BAS/ACD	K	HY+, BAS/ACD	K	E	R	
110	A	A	A	A	A	A	D	ALP/ACD	D	D	ALP/ACD	D	ALP/ACD	D	A		
111	T	T	T	T	T	T	T	T	T	T	T	T	T	T			
112	P	P	P	P	P	P	V	ALP	P	P	ALP	P	ALP	P	V		
113	E	E	E	E	E	E	E	E	E	E	E	Q	ACD	E	Q		
114	E	E	E	E	E	E	E	E	D	D	ACD	H	HY+, ACD/BAS	E	D	H	
115	T	T	T	T	T	T	T	T	T	T	T	S	ALH	T	S		
116	A	A	A	A	A	A	V	ALP	A	A	ALP	S	ALP/ALH	A	V	S	
117	Y	Y	Y	Y	Y	Y	F	ARO	F	F	ARO	A	ARO/ALP	Y	F	A	
118	D	D	D	D	D	D	N	ASX	E	E	ACD	D	ACD	D	E	N	
119	T	T	T	T	T	T	T	T	V	V	ALH/ALP	F	ALH/ALP/ARO	T	V	F	
120	F	F	F	F	F	F	F	F	T	T	ARO/ALH	F	ARO/ALH/ARO	F	T		
121	K	K	K	K	K	K	K	K	K	K	K	K	K	K			
122	C	C	V	C	C	HY-, SUL/ALP	C	HY- SUL/ALP	C	C	HY-, SUL/ALP	C	HY-, SUL/ALP	C	V	(3 4 is the only variant in the family)	
123	I	I	I	I	I	I	V	ALP	V	V	ALP	V	ALP	V	I		
124	Y	Y	Y	Y	Y	Y	M	ARO/SUL	L	L	ARO/SUL/ALP	H	ARO/SUL/ALP/BAS	Y	L	M	H
125	D	D	D	D	D	D	K	HY+ ACD/BAS	K	K	HY+, ACD/BAS	D	HY+, ACD/BAS	D	K		
126	S	S	S	S	S	S	N	HY+, ALH/ACD	D	D	HY+ ALH/ACD	N	HY+, ALH/ACD	S	N	D	
127	K	K	K	K	K	K	K	K	K	K	K	R	BAS	K	R		
128	P	P	P	P	P	P	P	P	P	P	P	S	ALP/ALH	P	S		
129	D	D	D	D	D	D	K	HY+ ACD/BAS	N	N	HY+ ACD/BAS		HY+, ACD/BAS OR GAP	D	N	K	
130	F	F	F	F	F	F	F	F	F	F	F		F OR GAP	F			
131	S	S	S	S	S	S	S	S	F	F	ALH/ARO		ALH/ARO OR GAP	S	F		
132	P	P	P	P	P	P	P	P	G	G	ALP		ALP OR GAP	P	G		
133	I	I	I	I	I	I	V	ALP	D	D	ALP/ACD		ALP/ACD OR GAP	I	D	V	
134	D	D	D	D	D	D	D	D	L	L	ACD/ALP		ACD/ALP OR GAP	D	L		
135									F	F	F, OR GAP		F OR GAP	F			
136									V	V	V, OR GAP		V, OR GAP	V			
137																	
138																	

FIG. 7.3 Cont.

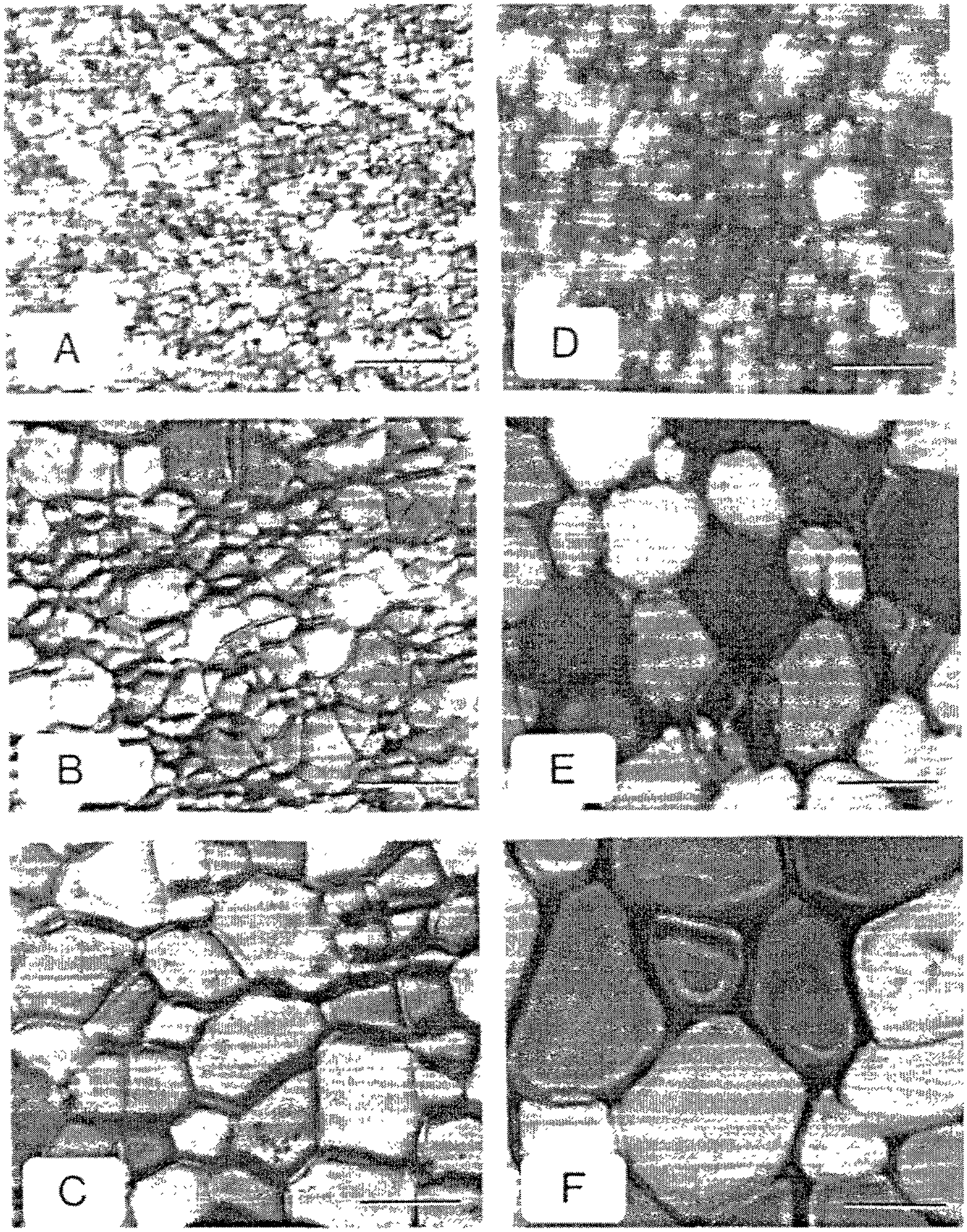


FIG. 8.0

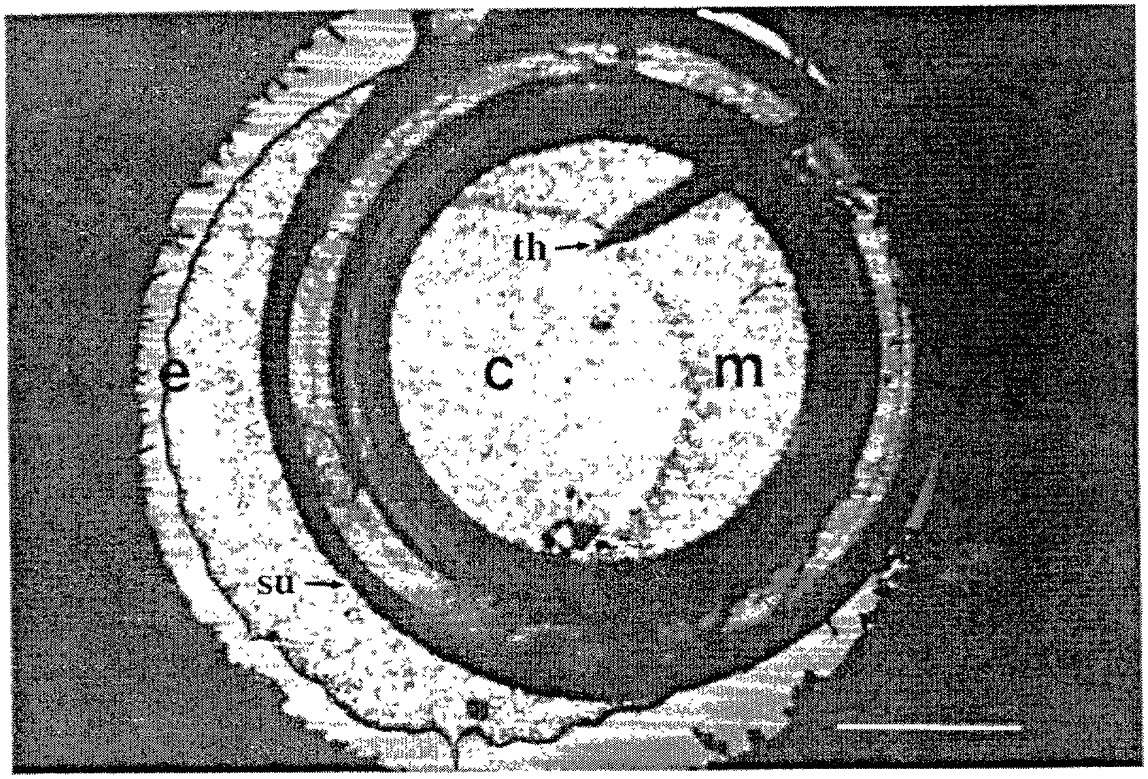


FIG. 8.1a

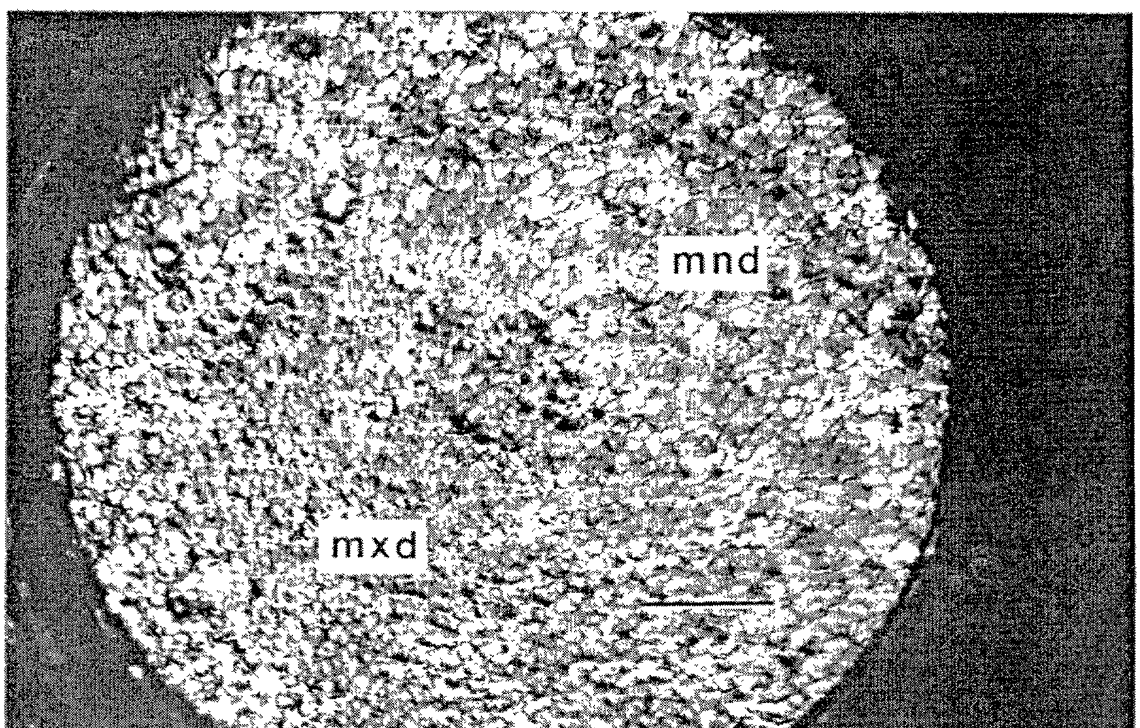


FIG. 8.1b

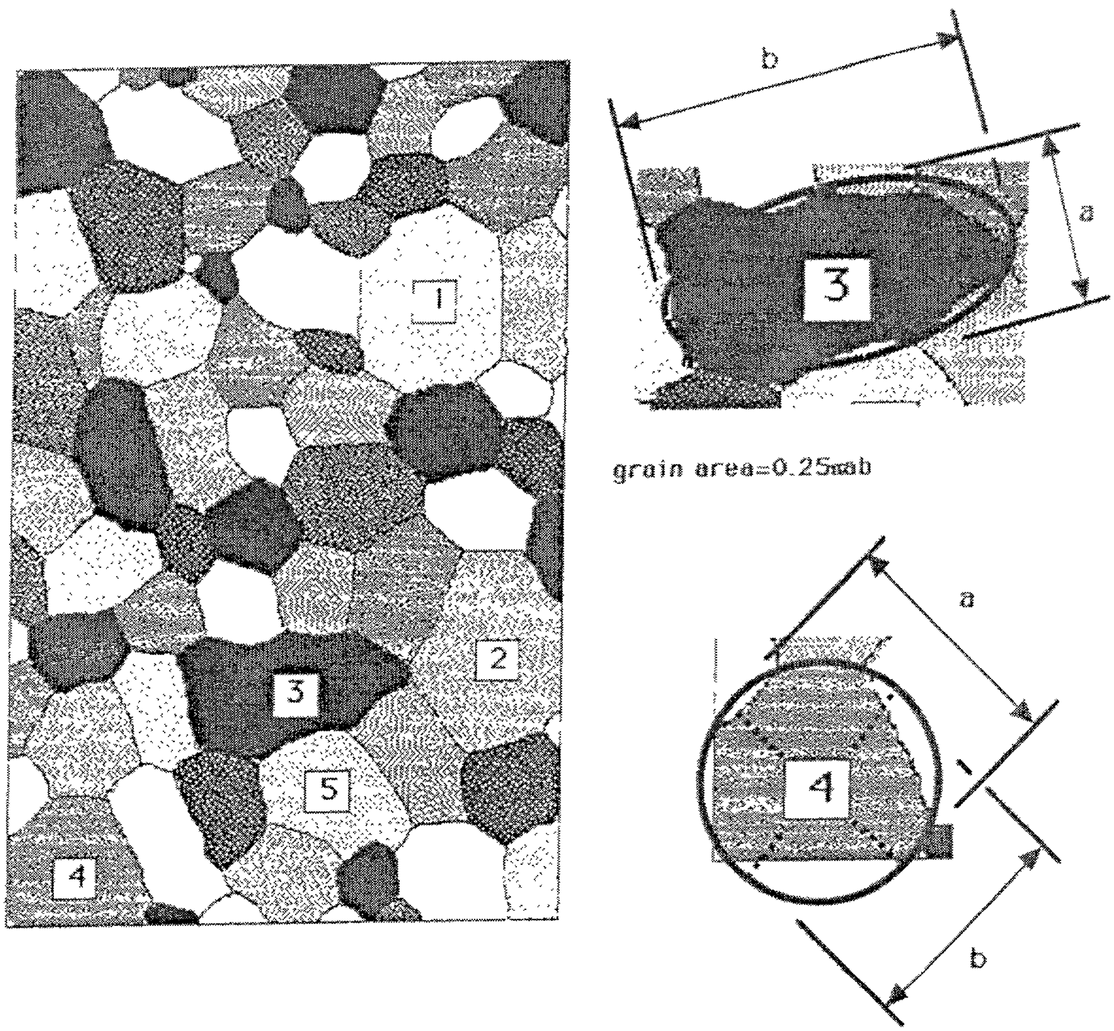


FIG. 8.2

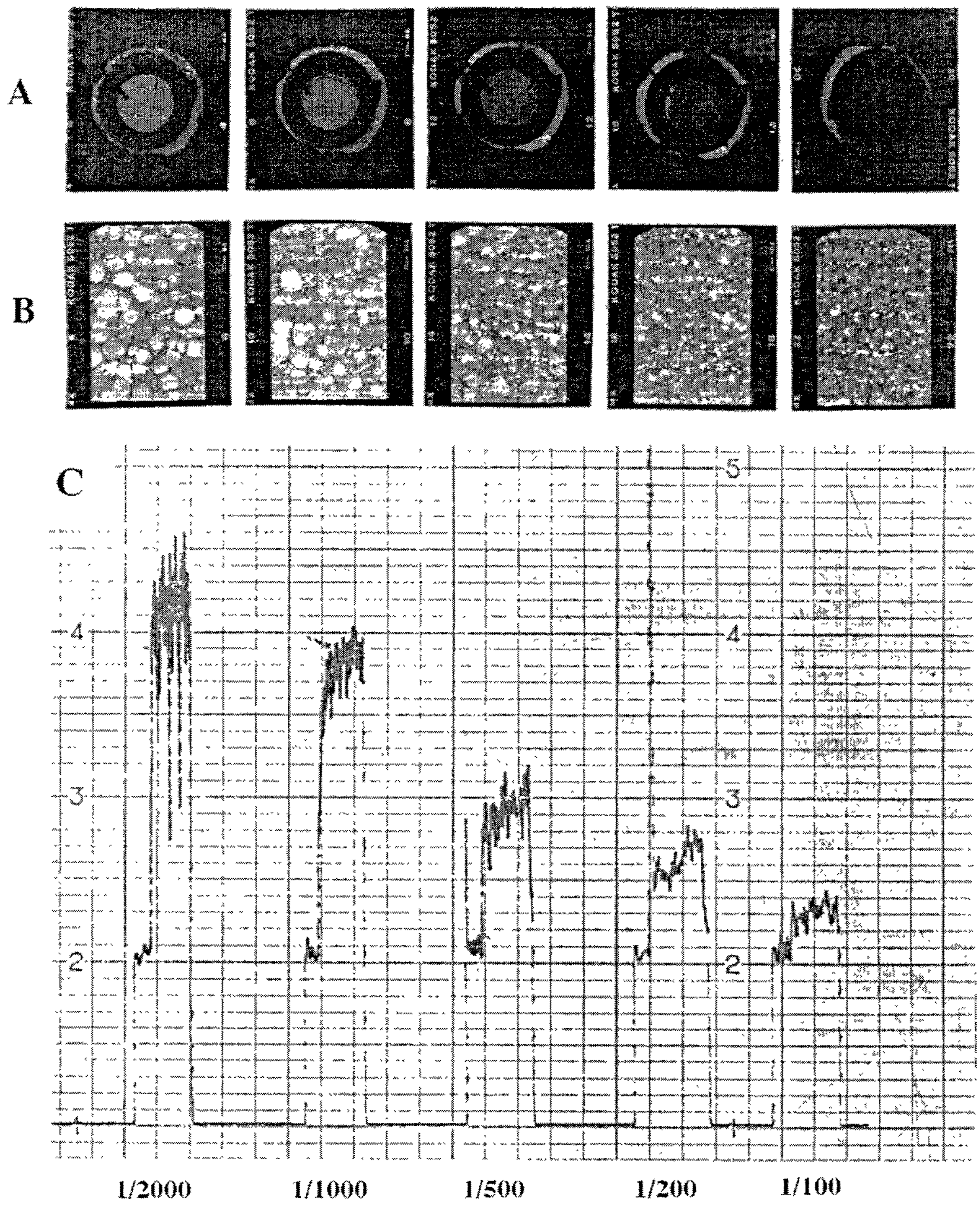
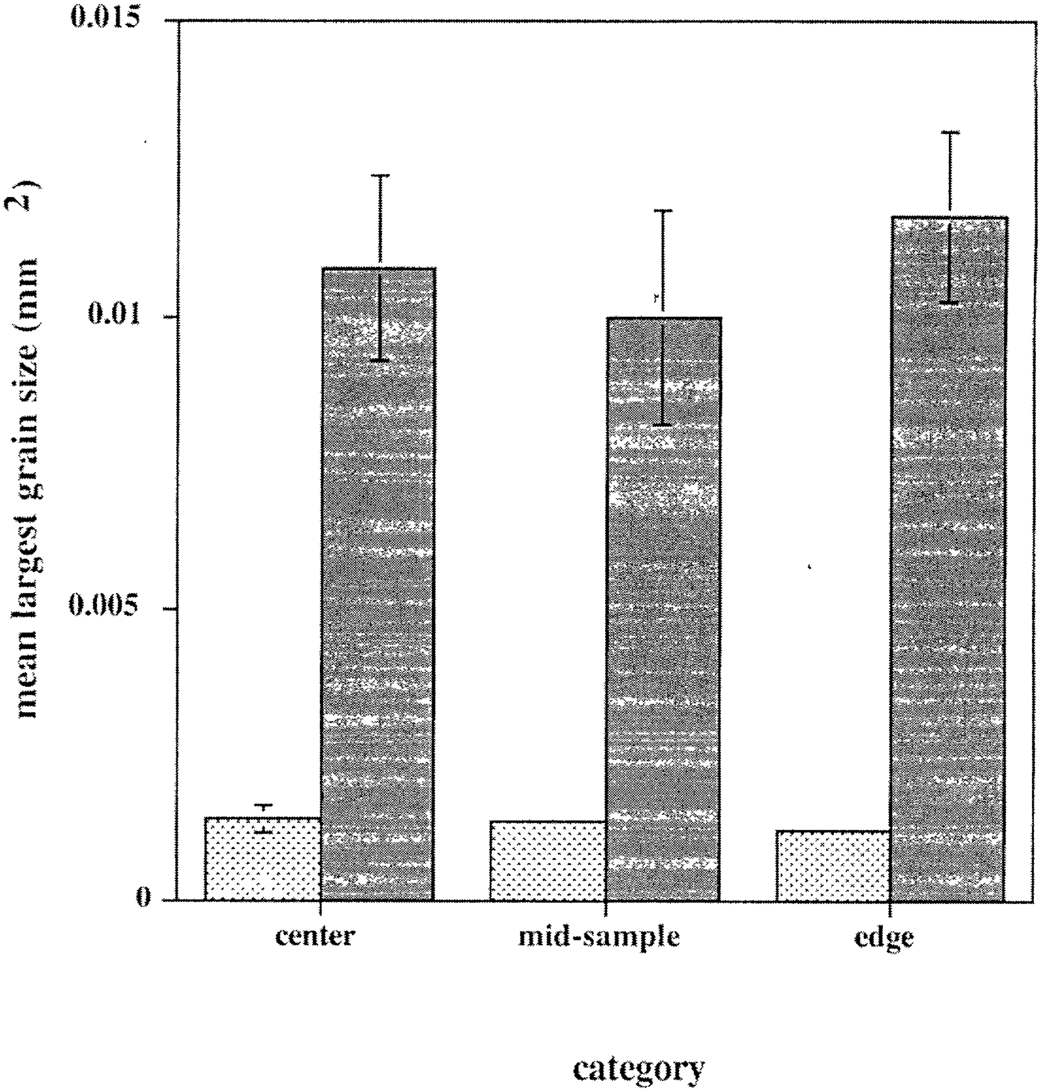


FIG. 8.3



0.015
0.01
0.005
0
mean largest grain size (mm 2)
center
mid-sample
edge
category

FIG. 8.4a

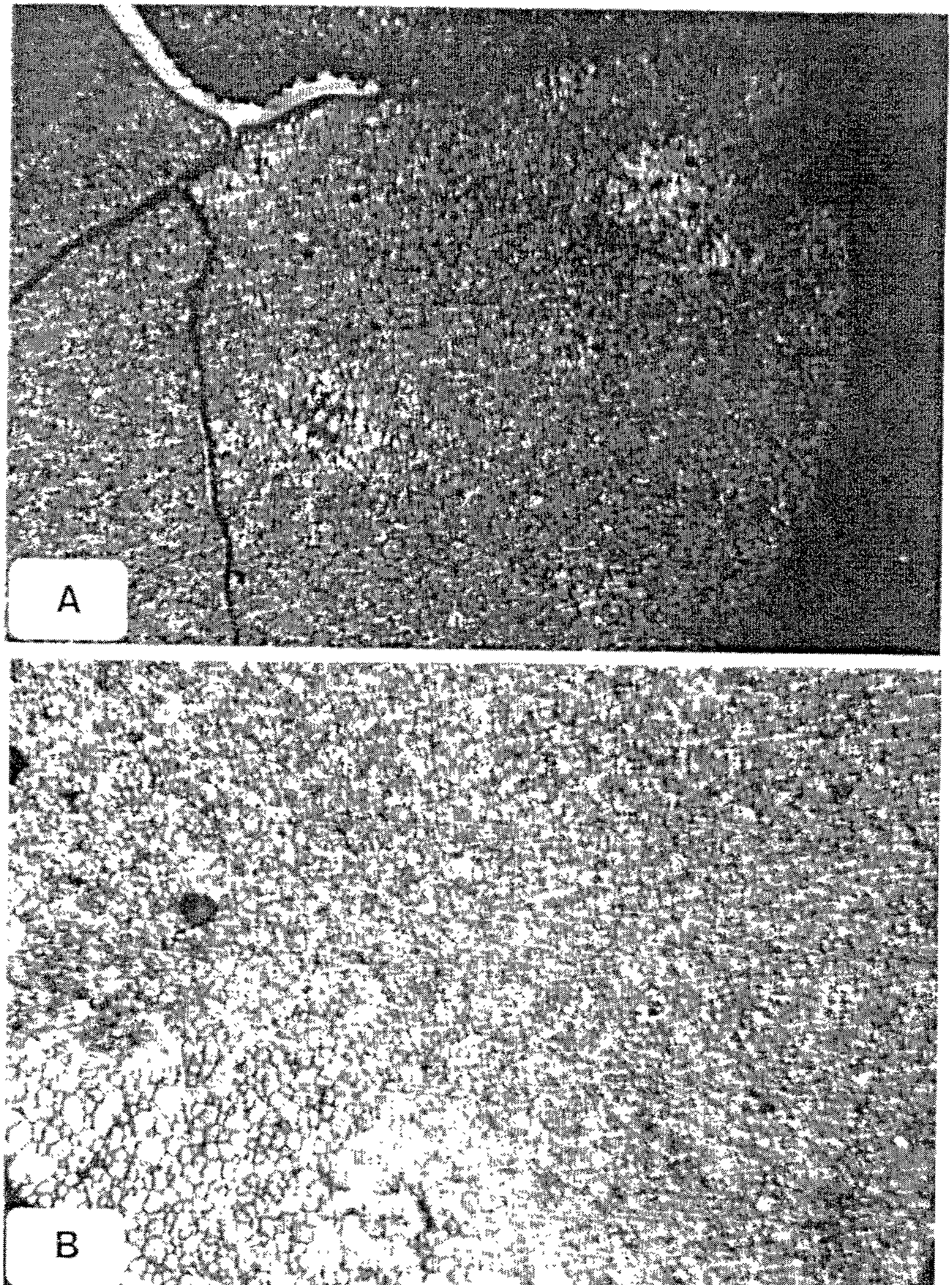


FIG. 8.4b

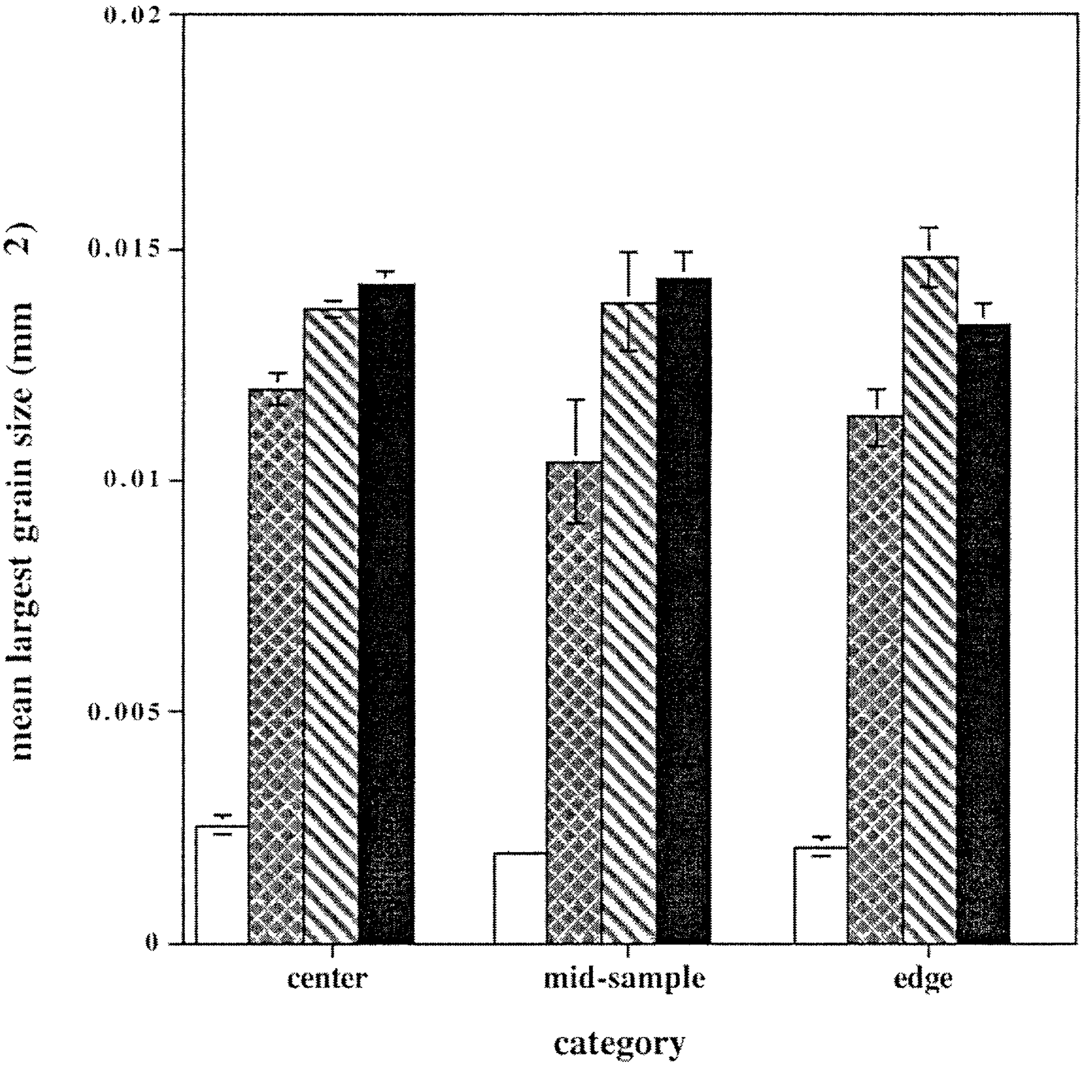

0.02
0.015
0.01
0.005
0
mean largest grain size (mm 2)
center
mid-sample
edge
category

FIG. 8.5a

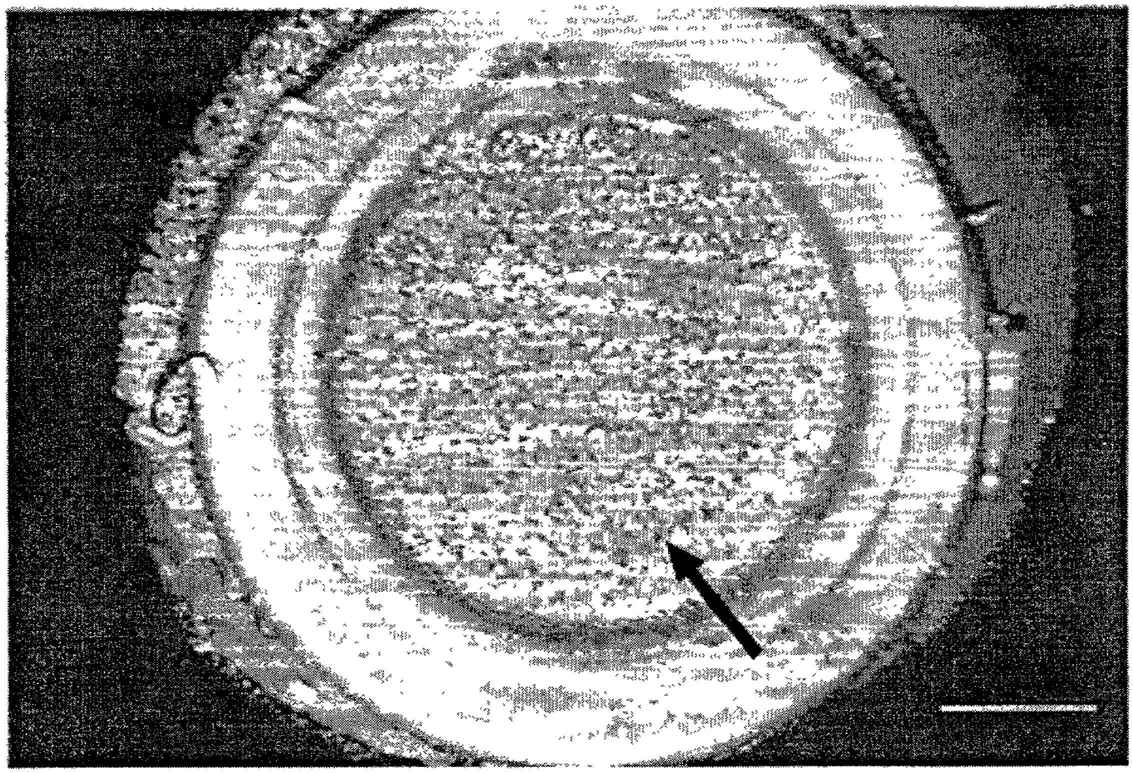


FIG. 8.5b

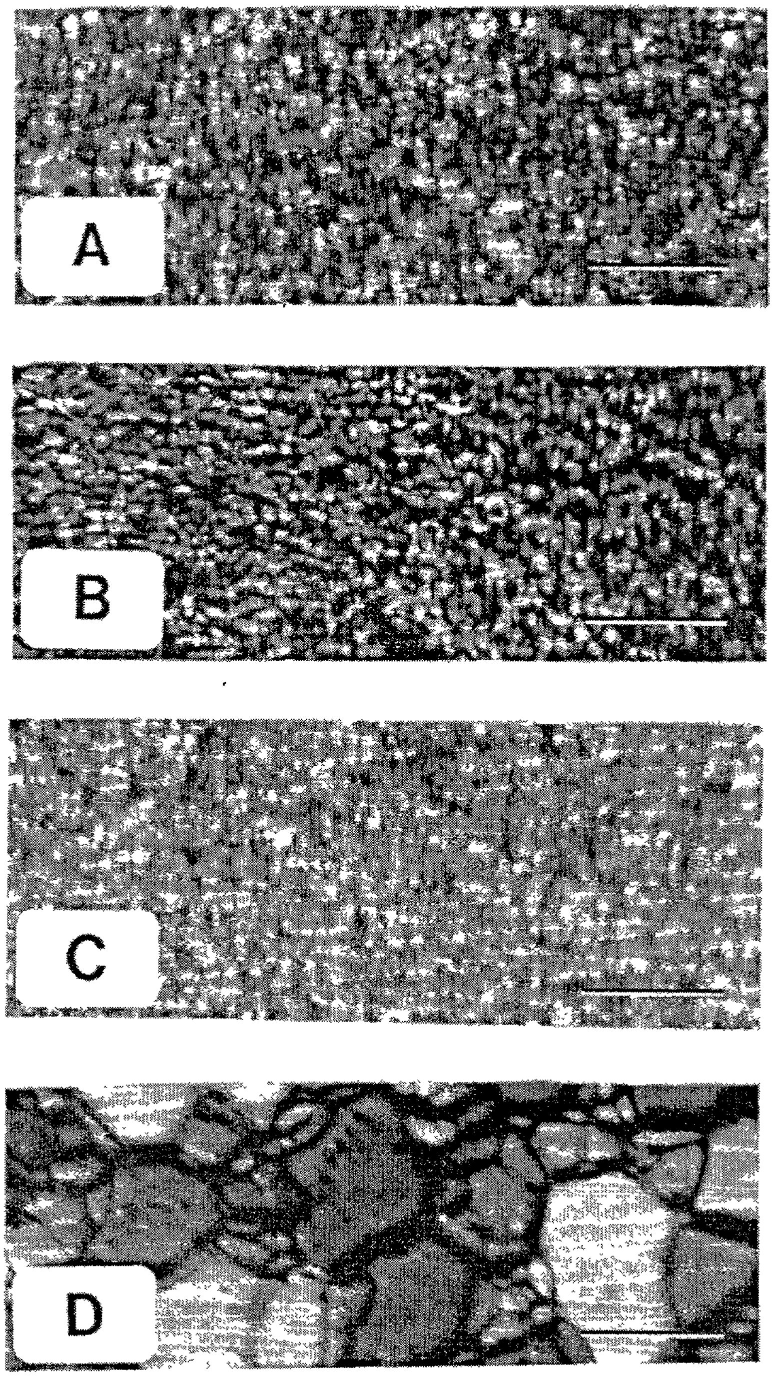


FIG. 8.6

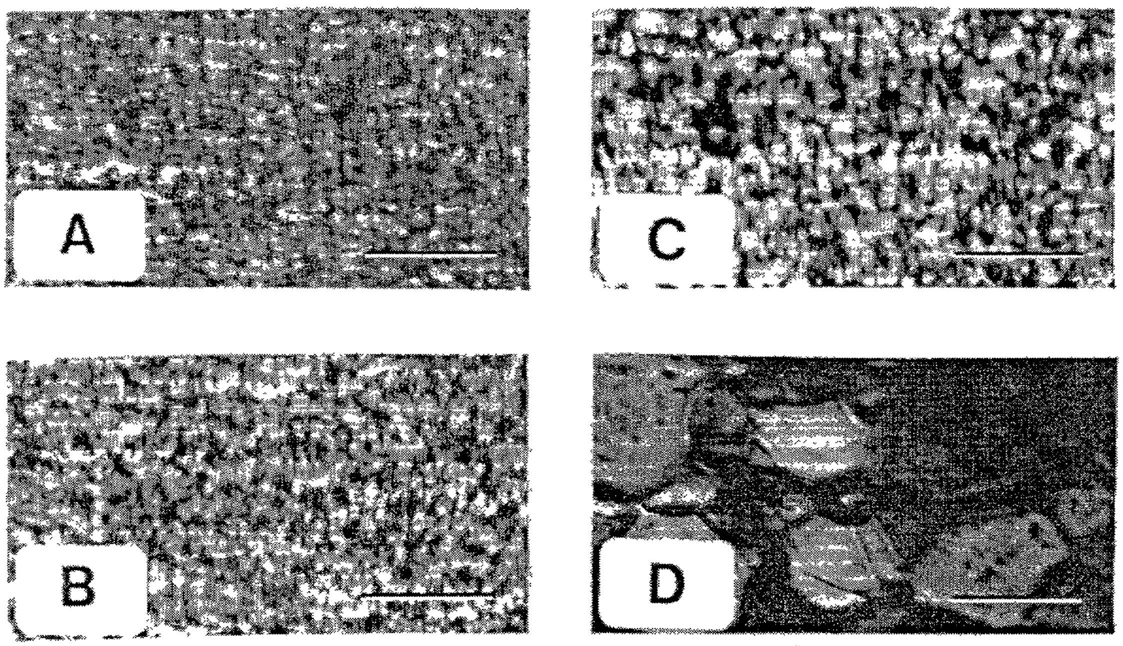
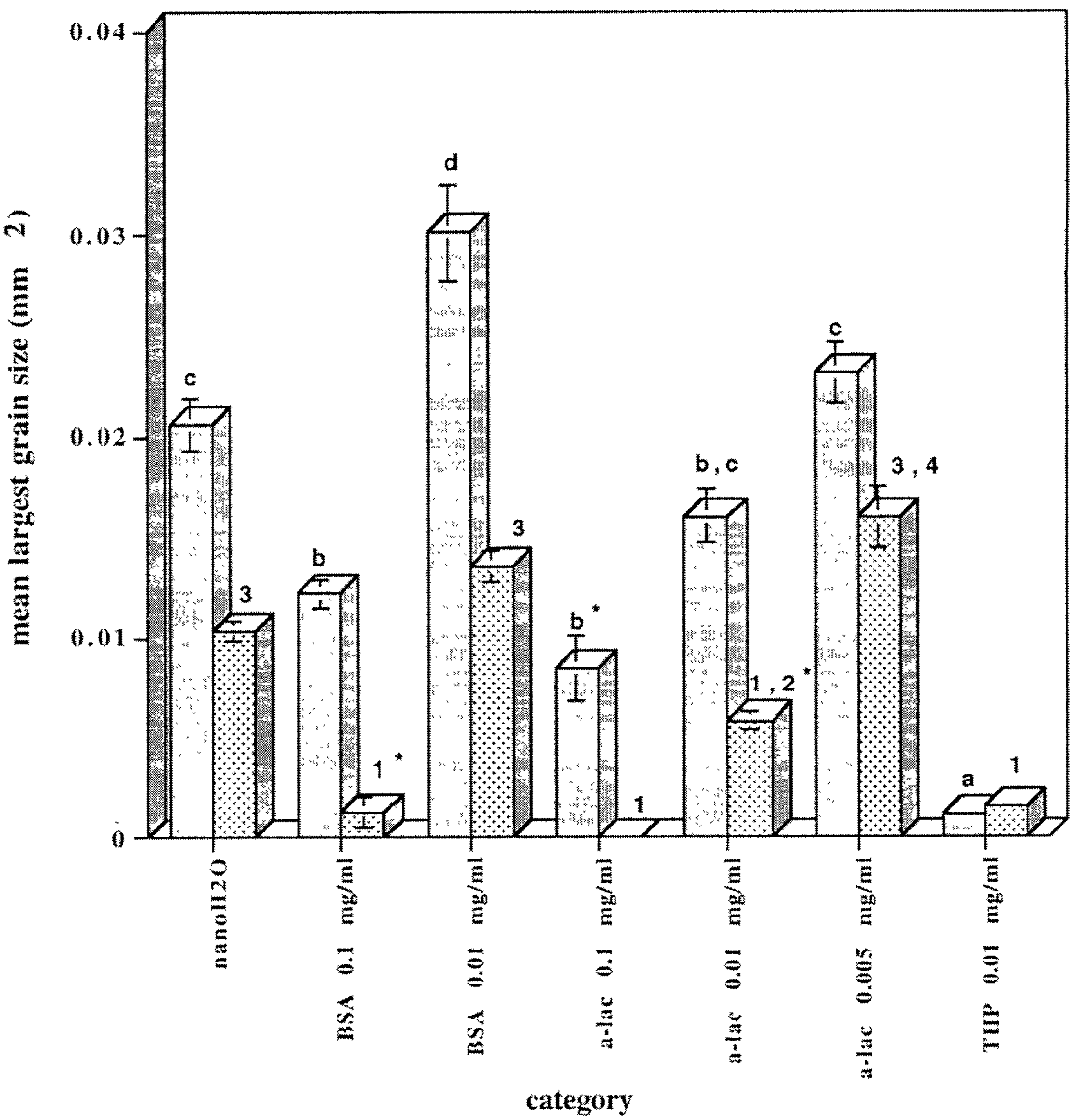


FIG. 8.7



0.04
0.03
0.02
0.01
0
mean largest grain size (mm 2)
c
3
b
1 *
d
3
b *
1
b , c
1 , 2 *
c
3 , 4
a
1
nanoH2O
BSA 0.1 mg/ml
BSA 0.01 mg/ml
a-lac 0.1 mg/ml
a-lac 0.01 mg/ml
a-lac 0.005 mg/ml
THP 0.01 mg/ml
category

FIG. 8.8

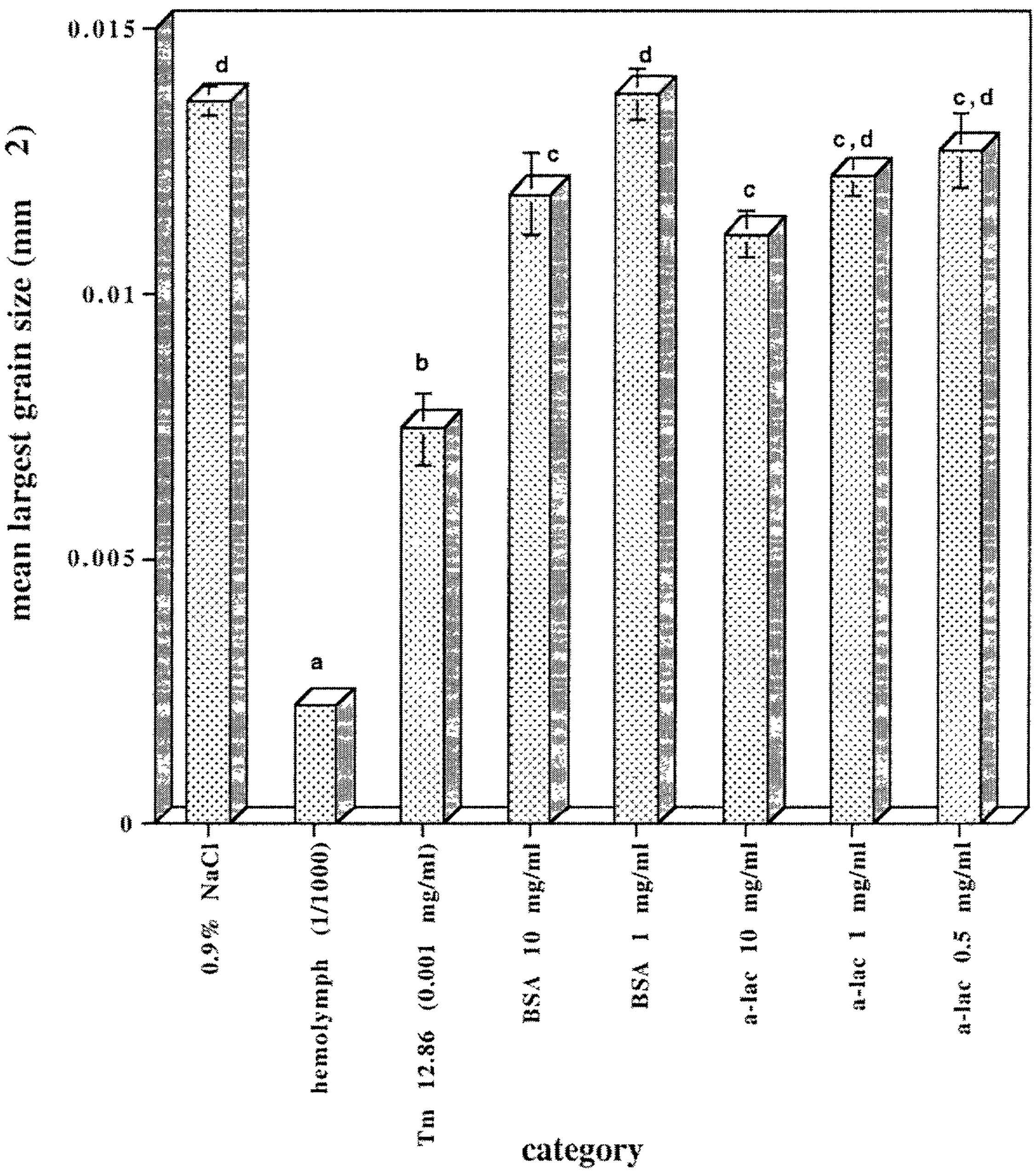
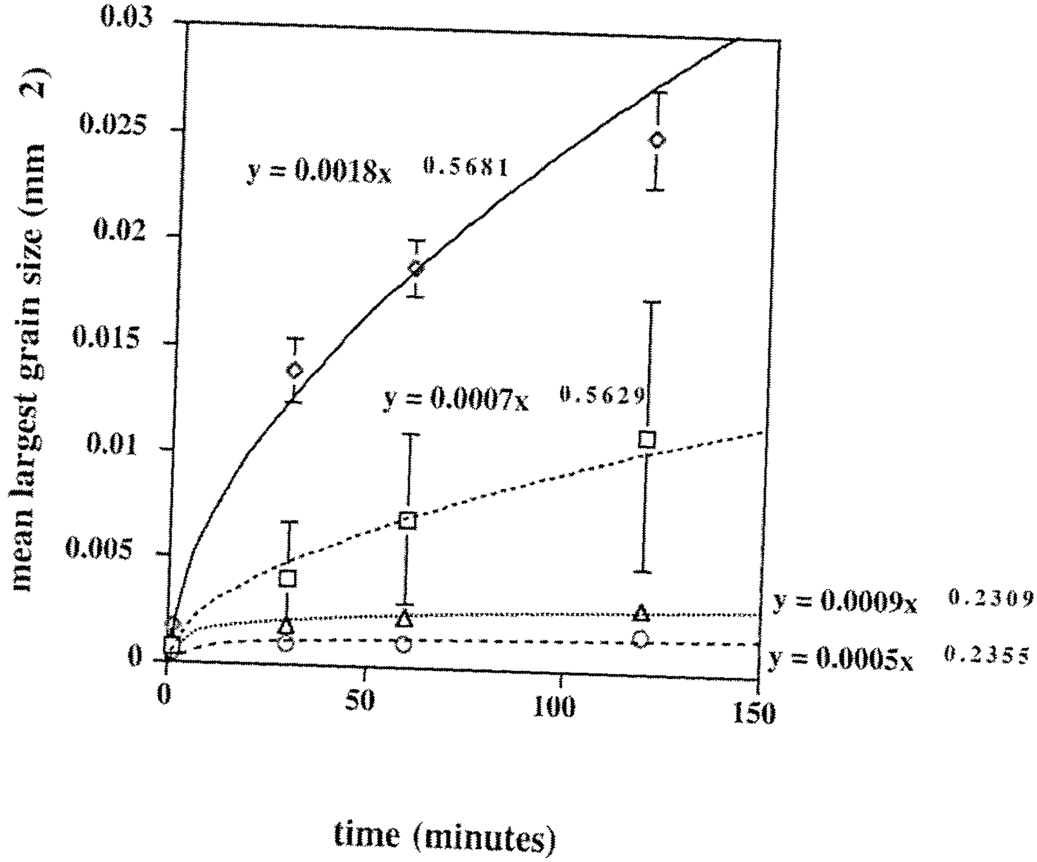
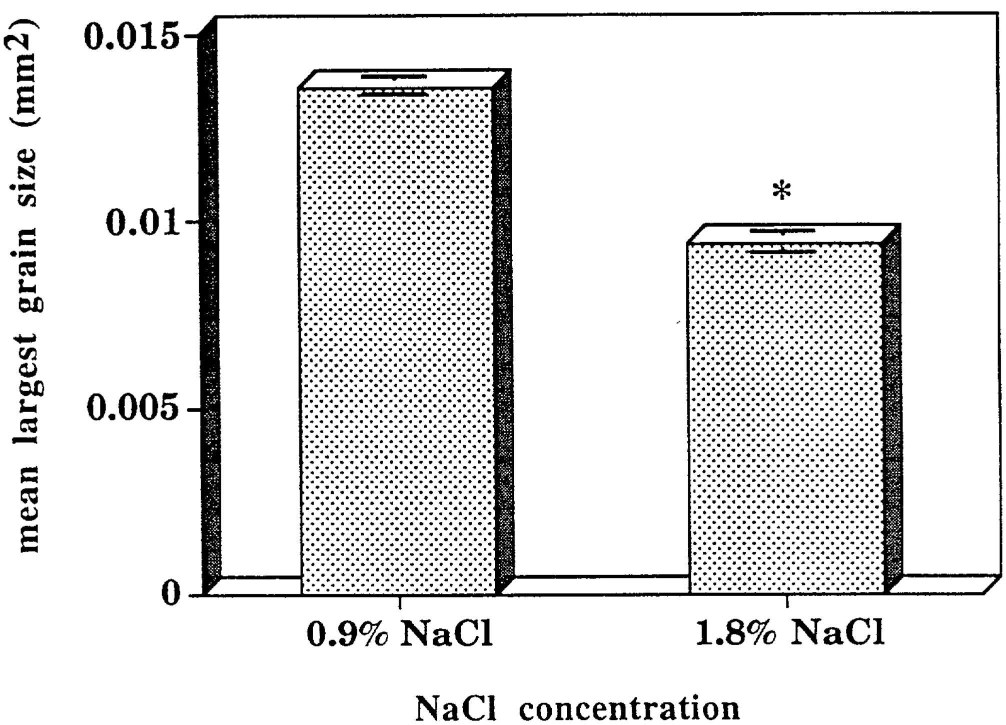

mean largest grain size (mm 2)
0.015
0.01
0.005
0
d
a
b
c
d
c
c, d
c, d
0.9% NaCl
hemolymph (1/1000)
Tm 12.86 (0.001 mg/ml)
BSA 10 mg/ml
BSA 1 mg/ml
a-lac 10 mg/ml
a-lac 1 mg/ml
a-lac 0.5 mg/ml
category

FIG. 8.9



0.03
0.025
0.02
0.015
0.01
0.005
0
mean largest grain size (mm 2)
y = 0.0018x 0.5681
y = 0.0007x 0.5629
y = 0.0009x 0.2309
y = 0.0005x 0.2355
0
50
100
150
time (minutes)

FIG. 8.10



mean largest grain size (mm2)
0.015
0.01
0.005
0
*
0.9% NaCl
1.8% NaCl
NaCl concentration

FIG. 8.11

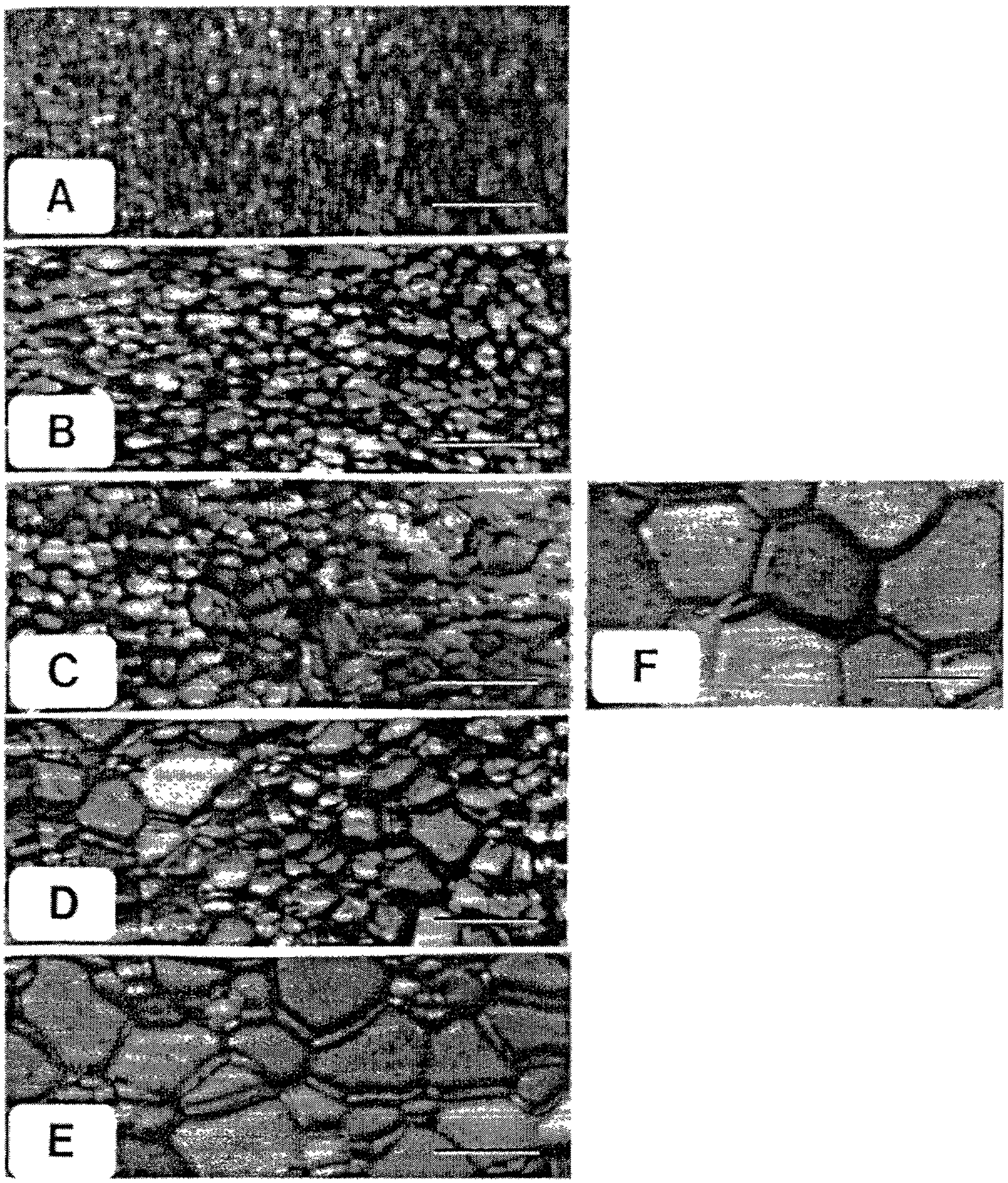
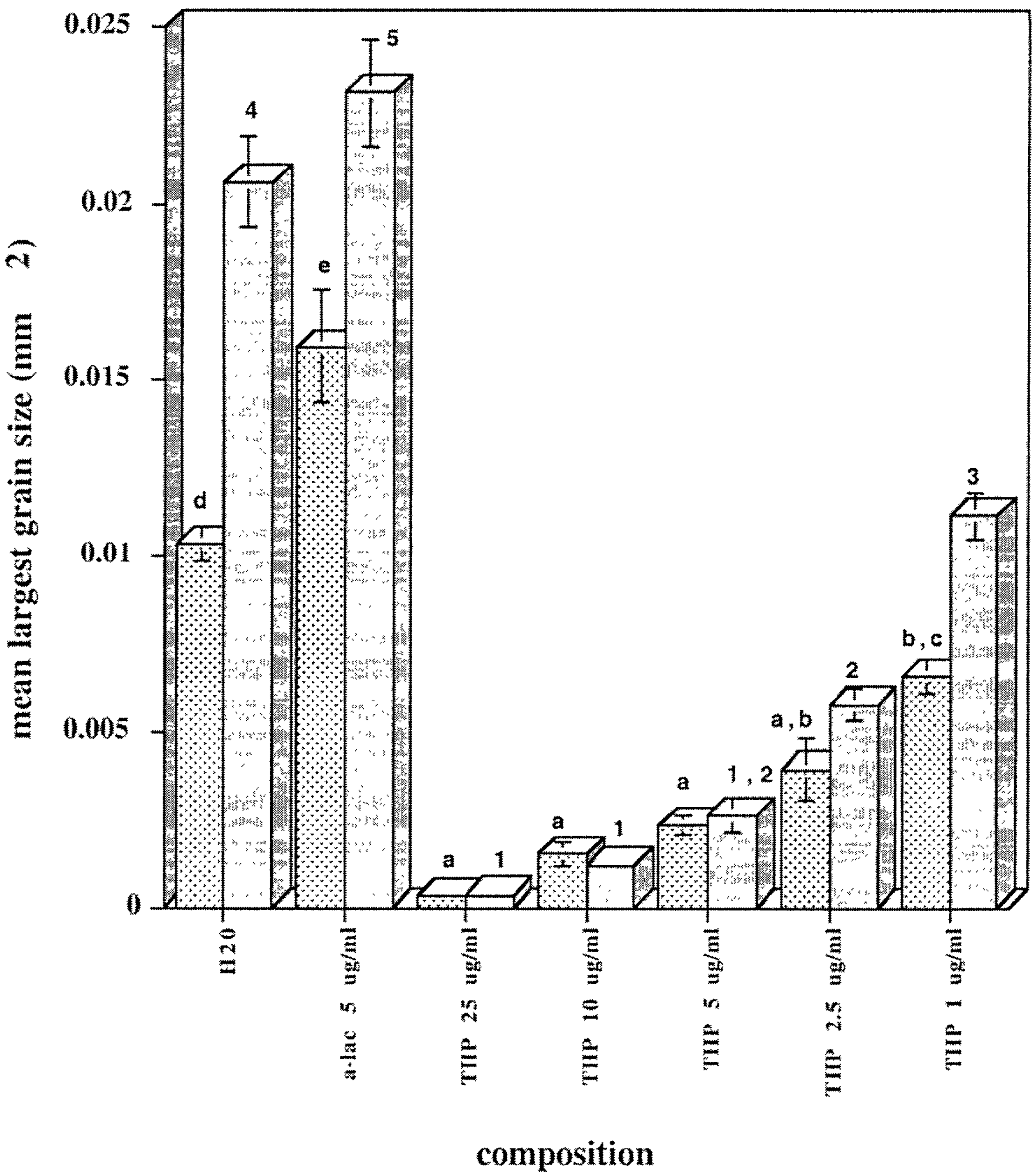


FIG. 8.12



0.025
0.02
0.015
0.01
0.005
0
mean largest grain size (mm 2)
d
4
e
5
a
1
a
1
a
1 , 2
a , b
2
b , c
3
H20
a-lac 5 ug/ml
THP 25 ug/ml
THP 10 ug/ml
THP 5 ug/ml
THP 2.5 ug/ml
THP 1 ug/ml
composition

FIG. 8.13

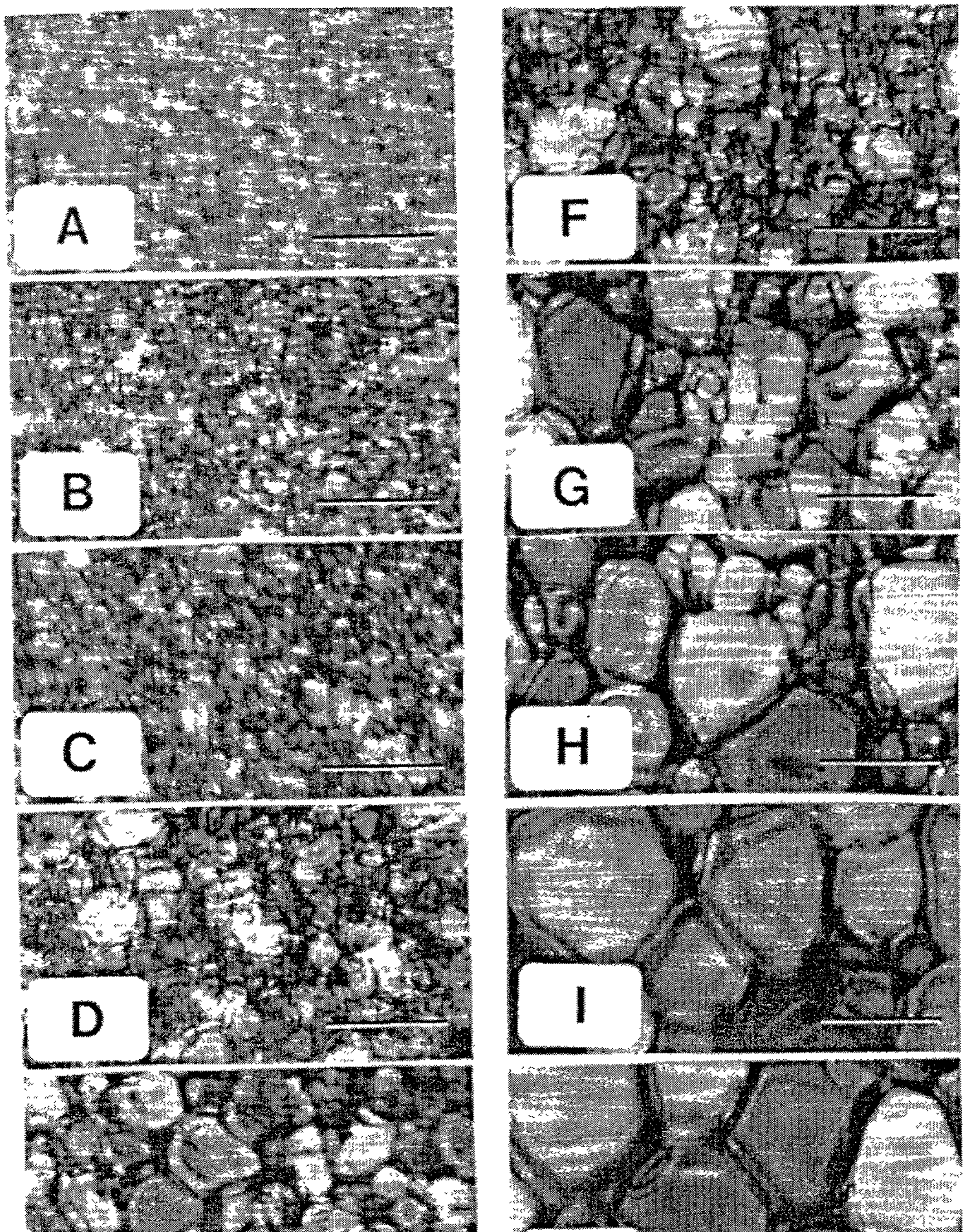


FIG. 8.14

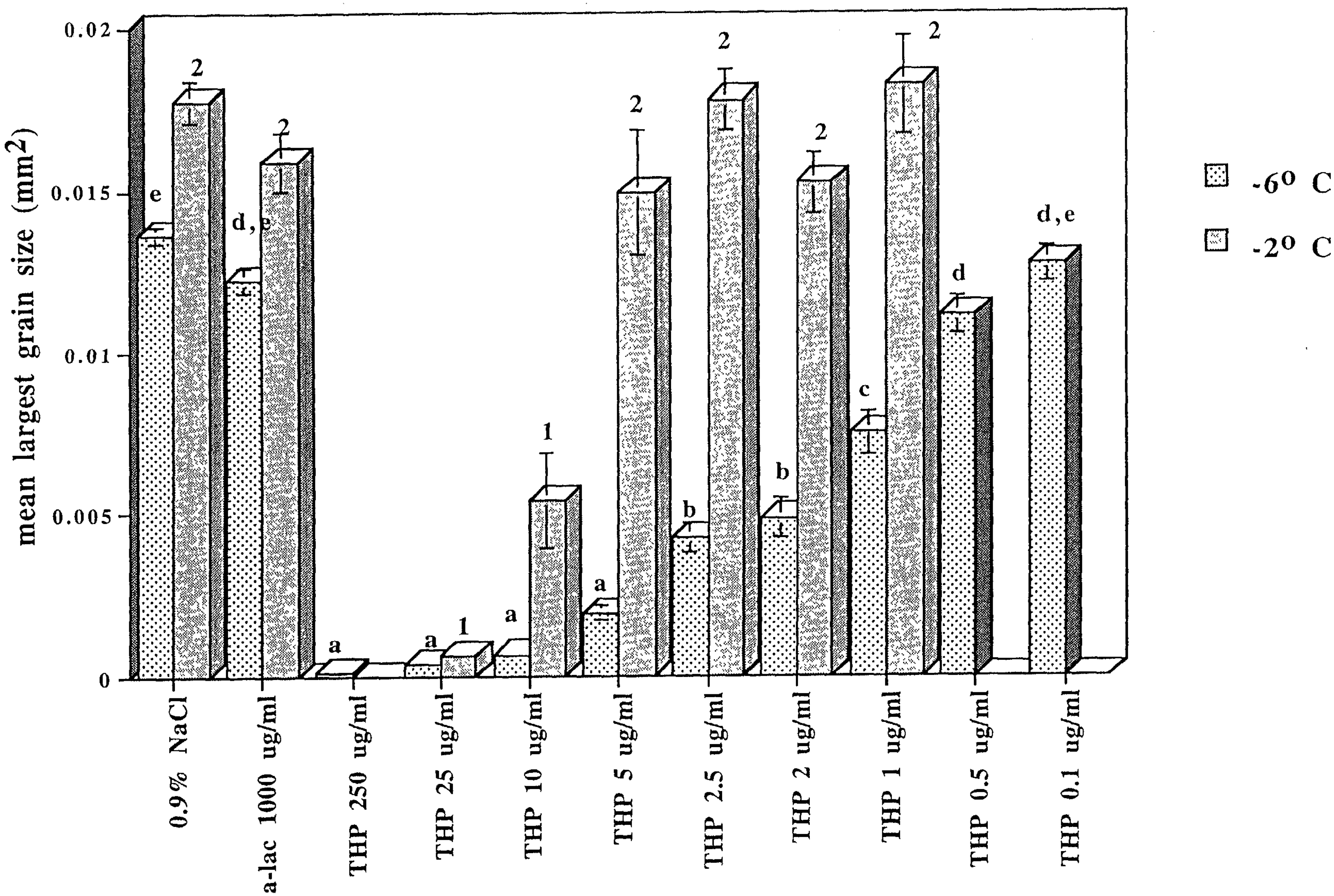

mean largest grain size (mm²)
0.02
0.015
0.01
0.005
0
0.9% NaCl
a-lac 1000 ug/ml
THP 250 ug/ml
THP 25 ug/ml
THP 10 ug/ml
THP 5 ug/ml
THP 2.5 ug/ml
THP 2 ug/ml
THP 1 ug/ml
THP 0.5 ug/ml
THP 0.1 ug/ml
e
2
d, e
a
1
b
c
d
-6° C
-2° C

FIG. 8.15

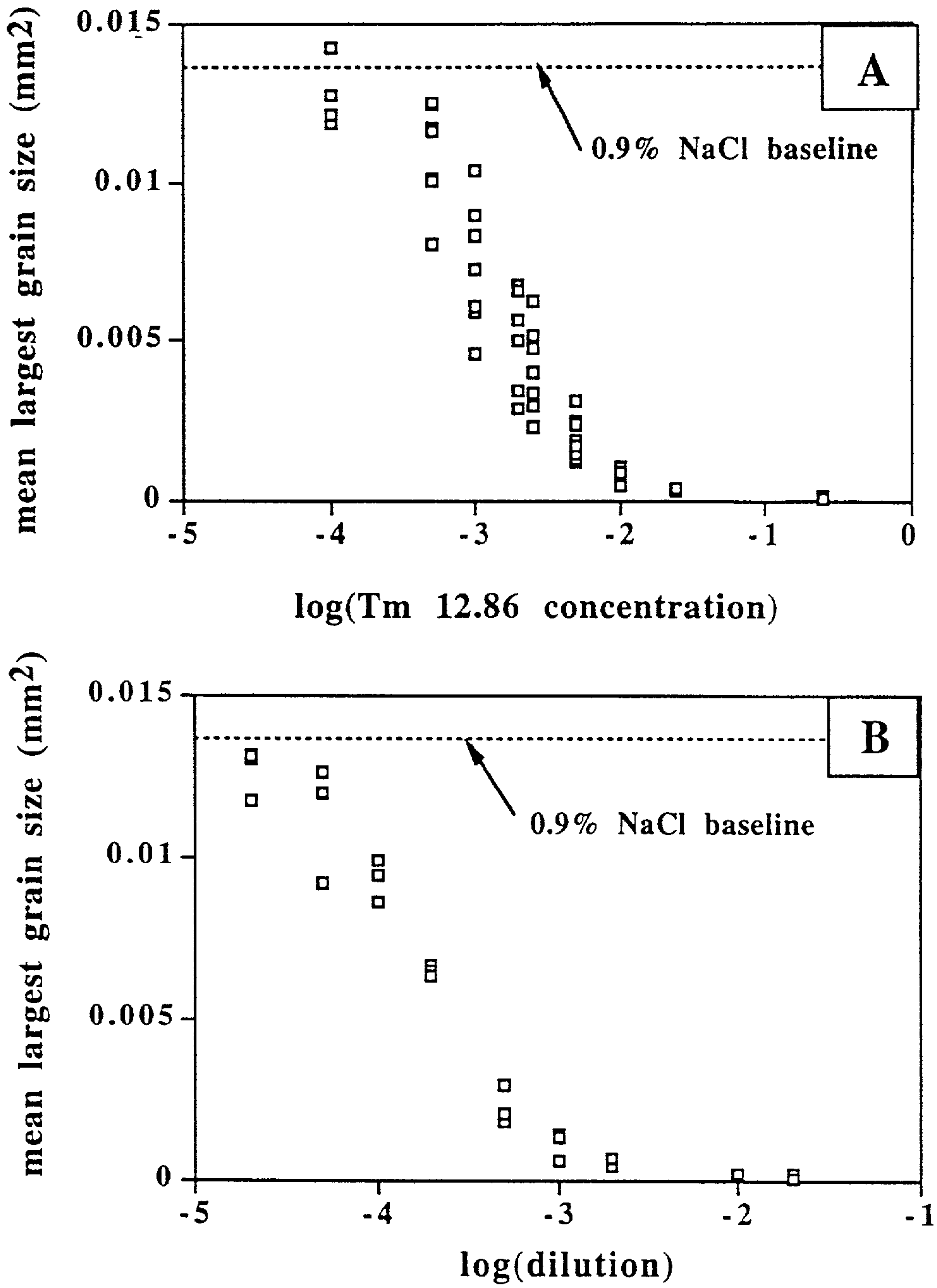

A
0.9% NaCl baseline
mean largest grain size (mm2)
0.015
0.01
0.005
0
-5
-4
-3
-2
-1
0
log(Tm 12.86 concentration)
B
0.9% NaCl baseline
mean largest grain size (mm2)
0.015
0.01
0.005
0
-5
-4
-3
-2
-1
log(dilution)

FIG. 8.16

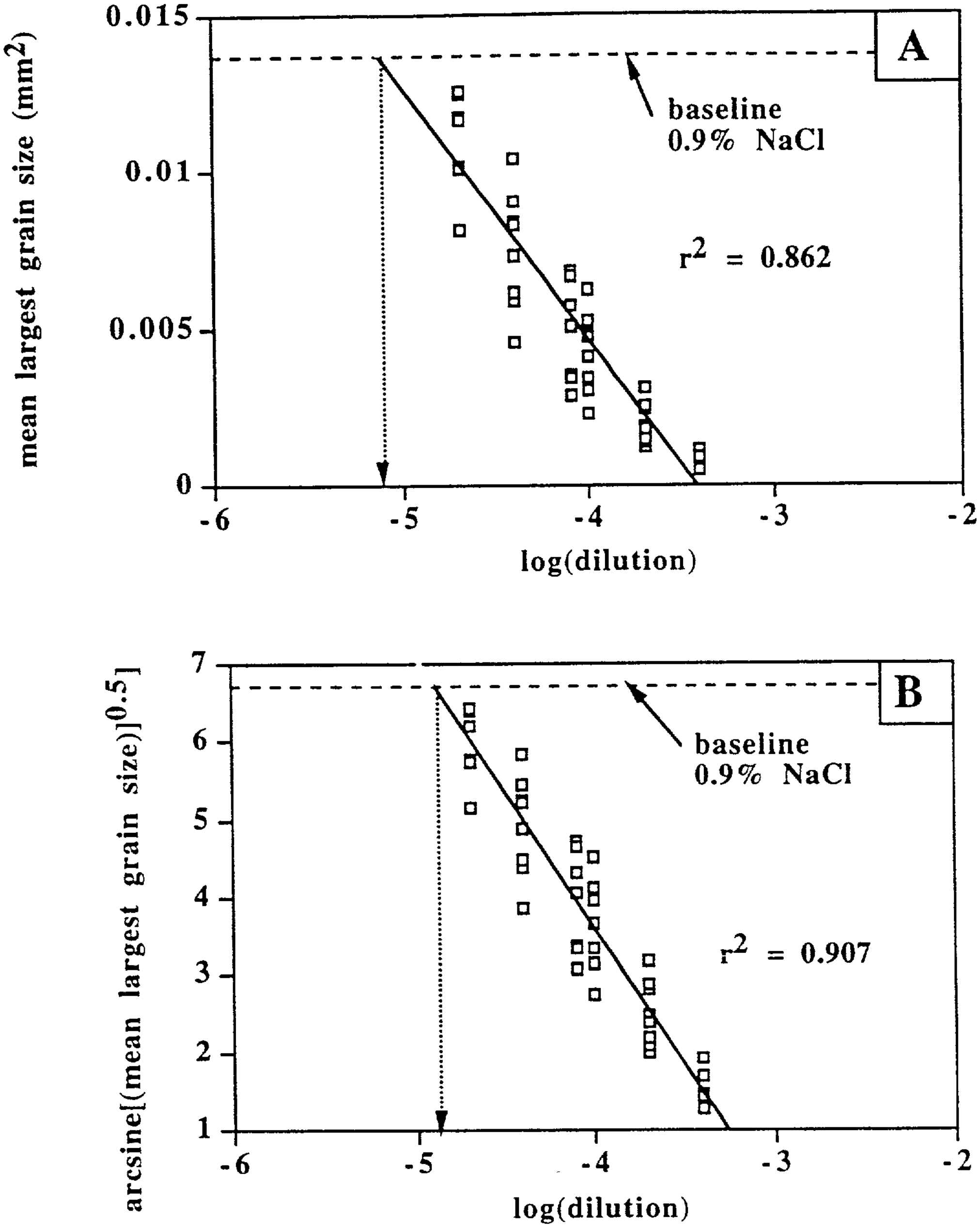
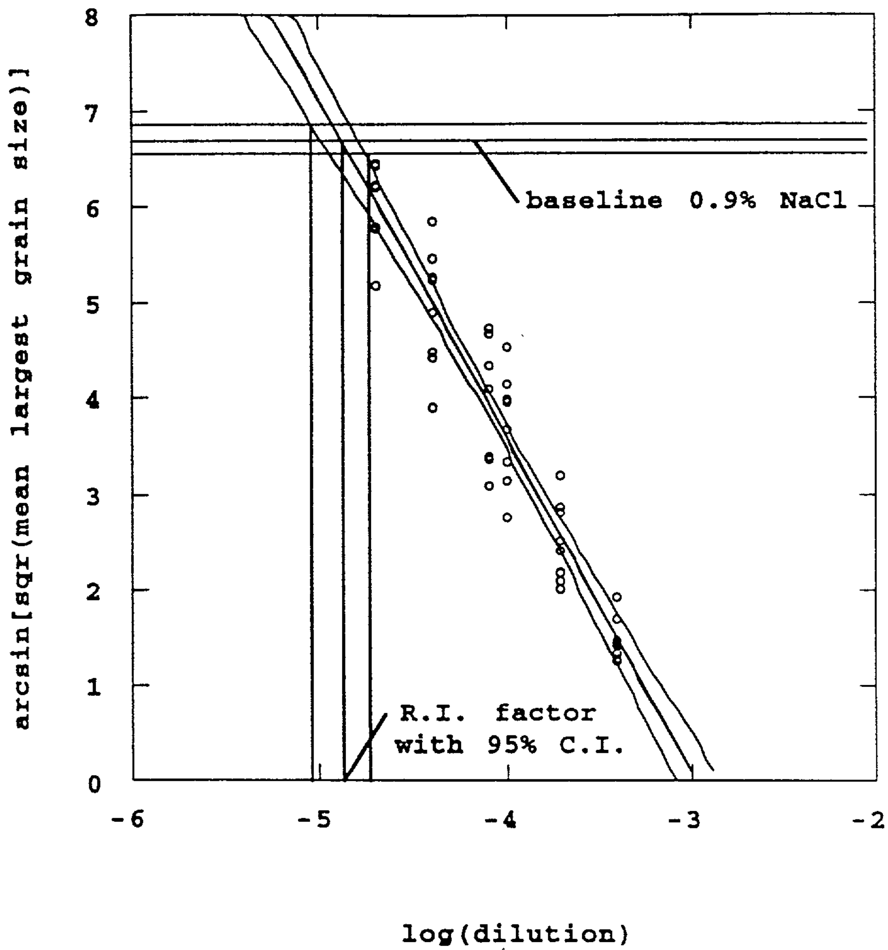

A
0.015
0.01
0.005
0
mean largest grain size (mm2)
baseline
0.9% NaCl
r2 = 0.862
-6
-5
-4
-3
-2
log(dilution)
B
7
6
5
4
3
2
1
arcsine[(mean largest grain size)]0.5]
baseline
0.9% NaCl
r2 = 0.907
-6
-5
-4
-3
-2
log(dilution)

FIG. 8.17



8
7
6
5
4
3
2
1
0
-6
-5
-4
-3
-2
arcsin[sqr(mean largest grain size)]
log(dilution)
baseline 0.9% NaCl
R.I. factor
with 95% C.I.

FIG. 8.18

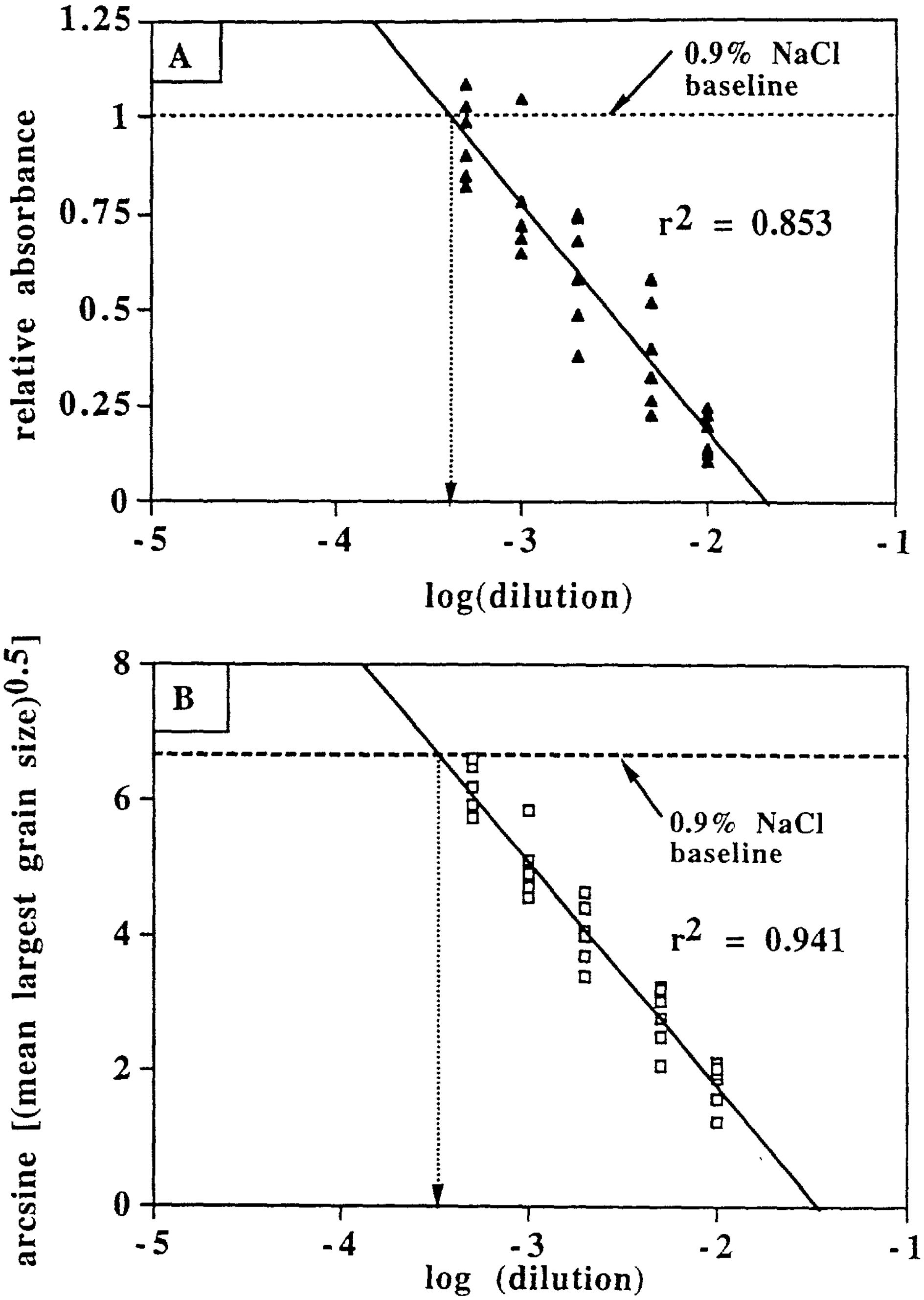

A
0.9% NaCl
baseline
r2 = 0.853
relative absorbance
1.25
1
0.75
0.5
0.25
0
-5
-4
-3
-2
-1
log(dilution)
B
0.9% NaCl
baseline
r2 = 0.941
arcsine [(mean largest grain size)0.5]
8
6
4
2
0
-5
-4
-3
-2
-1
log (dilution)

FIG. 8.19

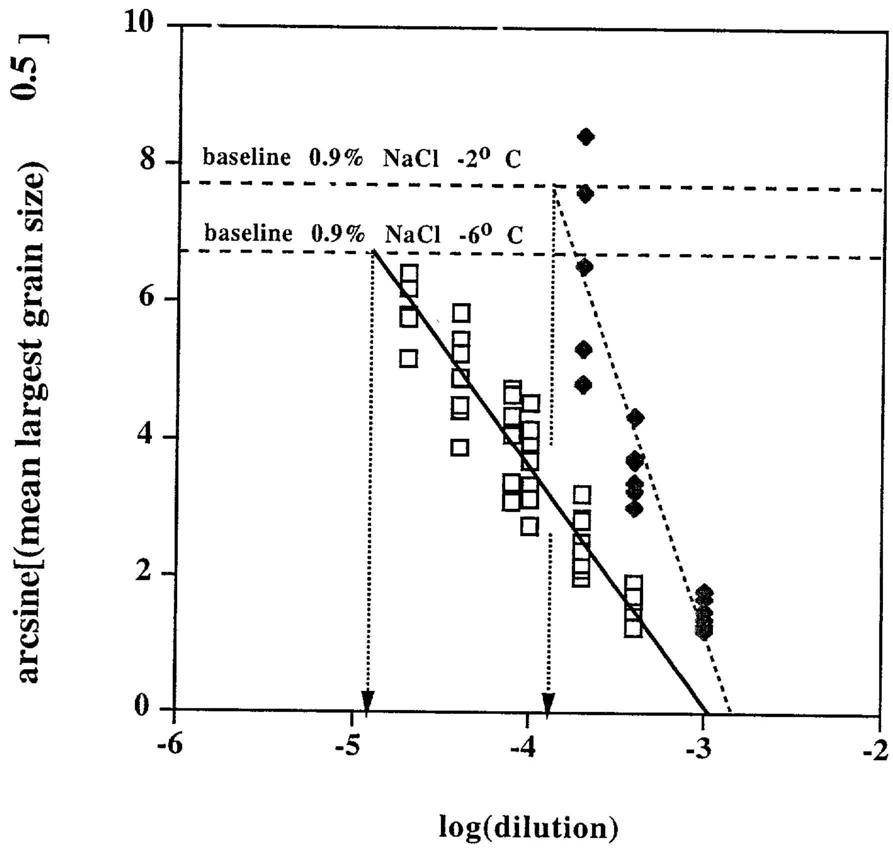
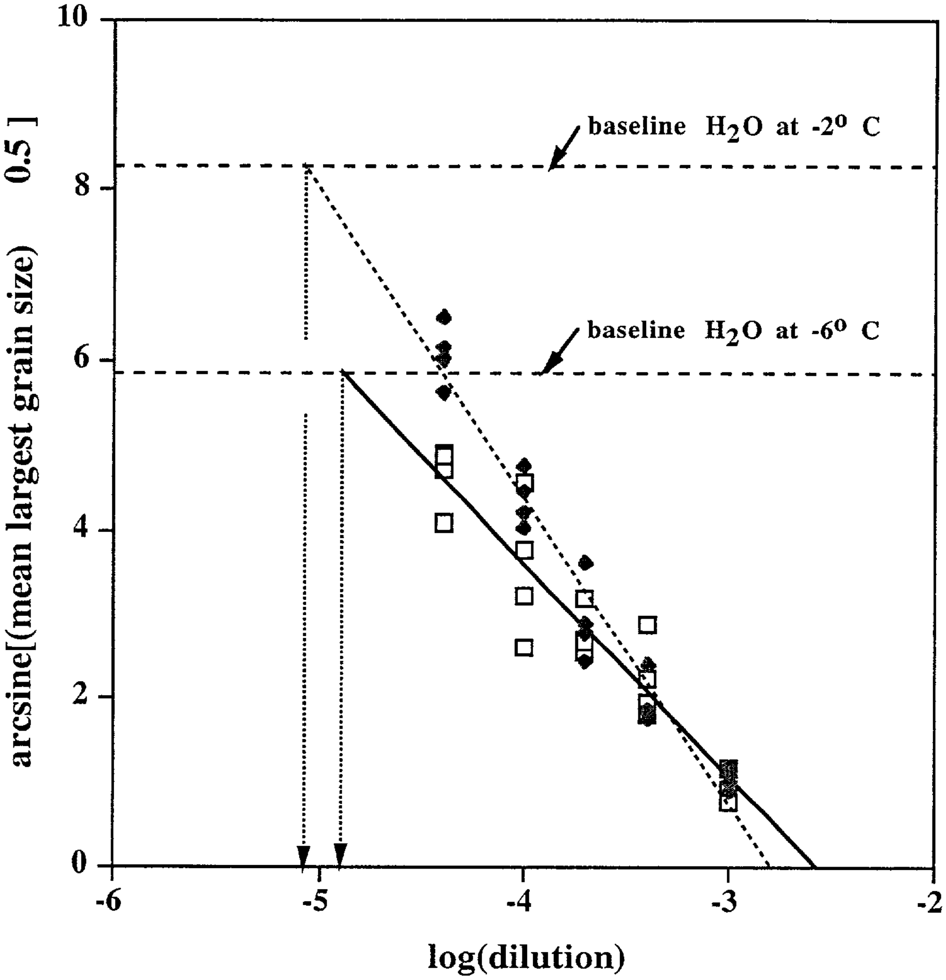

10
8
6
4
2
0
-6
-5
-4
-3
-2
baseline 0.9% NaCl -2° C
baseline 0.9% NaCl -6° C
arcsine[(mean largest grain size) 0.5]
log(dilution)

FIG. 8.20



10
8
6
4
2
0
arcsine[(mean largest grain size) 0.5]
baseline H_2O at -2° C
baseline H_2O at -6° C
-6
-5
-4
-3
-2
log(dilution)

FIG. 8.21

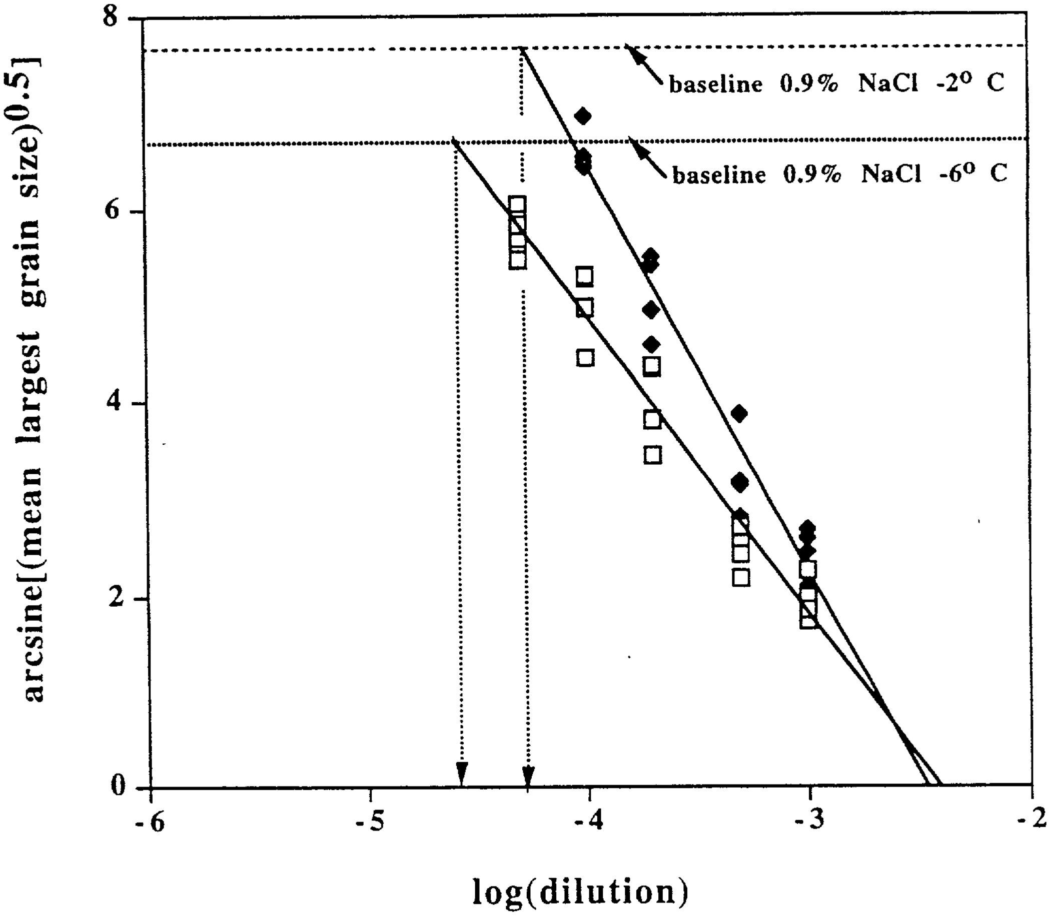

baseline 0.9% NaCl -2° C
baseline 0.9% NaCl -6° C
arcsine[(mean largest grain size)0.5]
log(dilution)
8
6
4
2
0
-6
-5
-4
-3
-2

FIG. 8.22

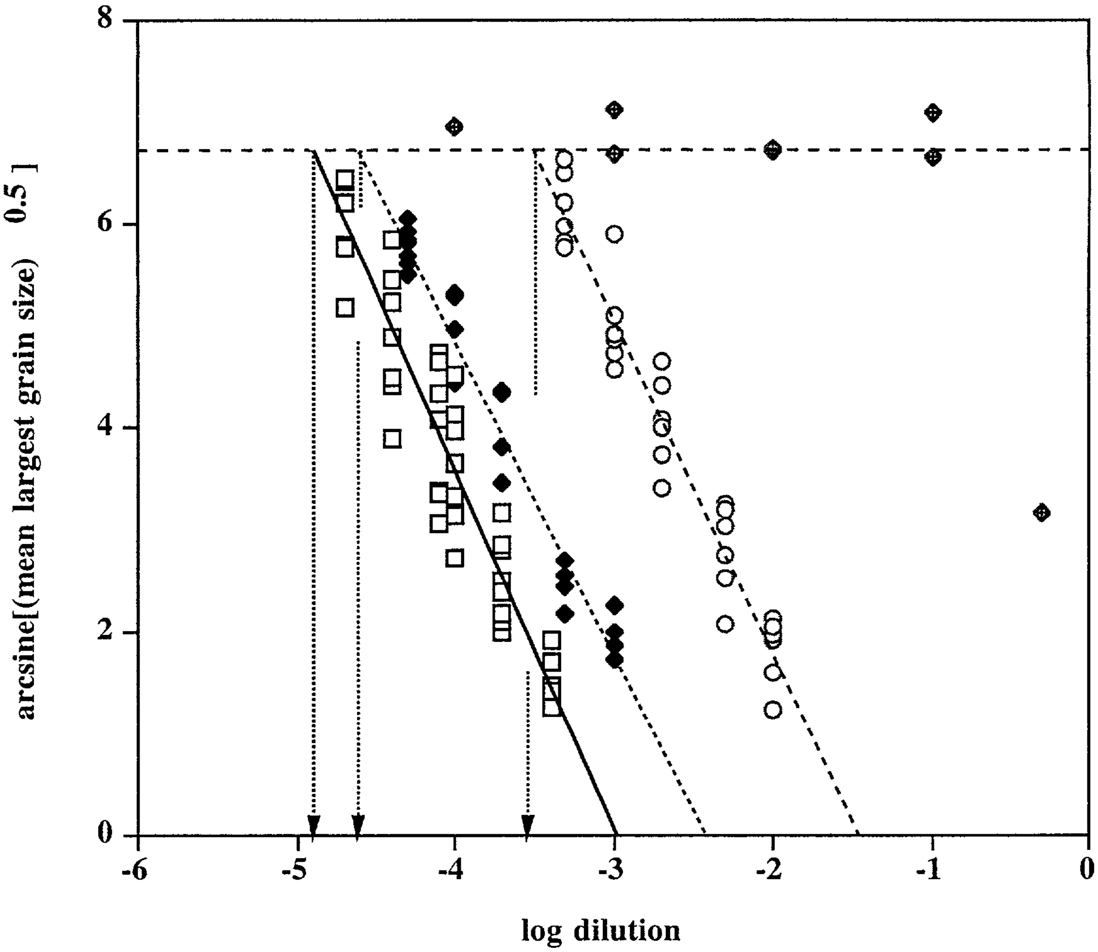
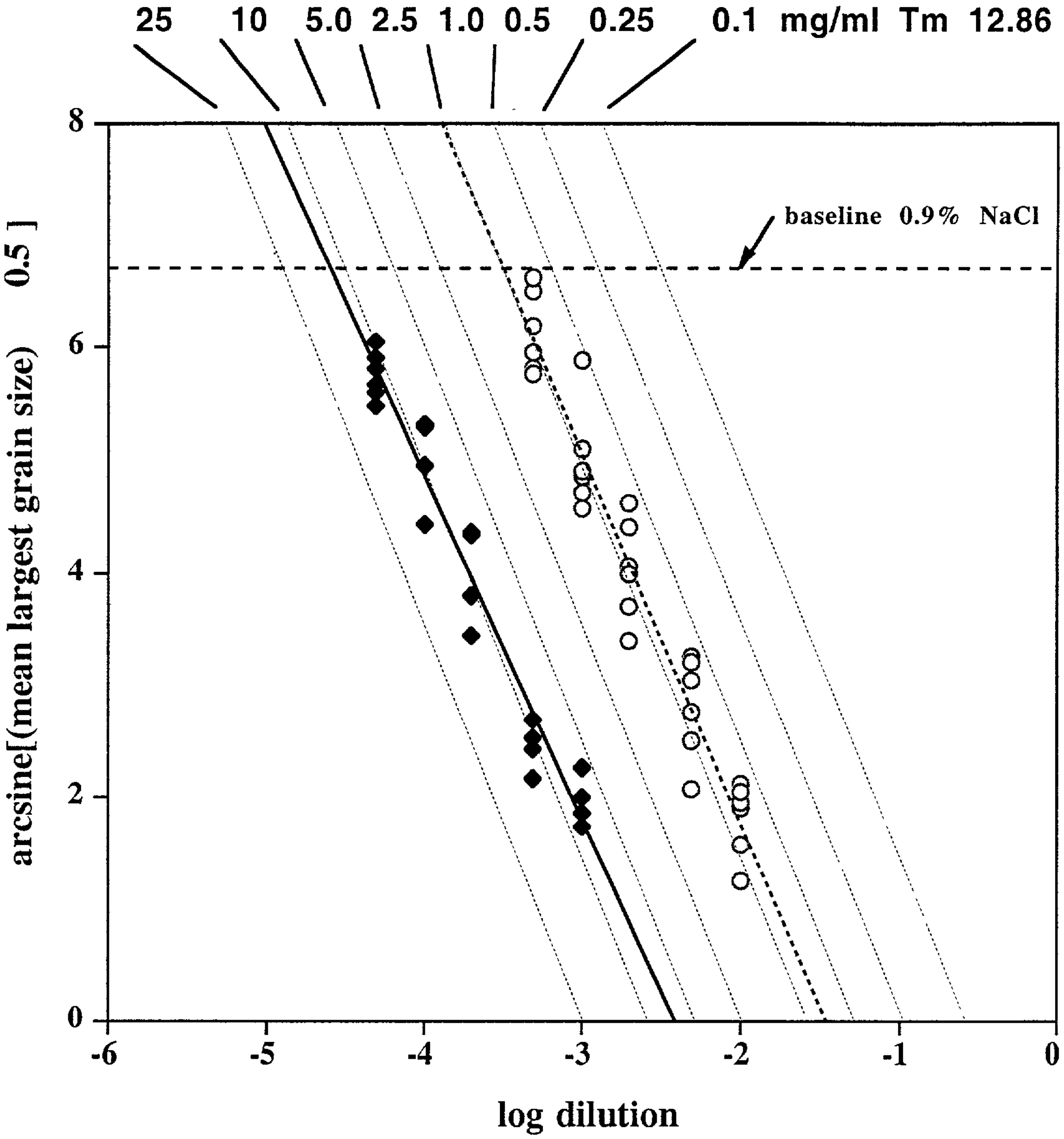
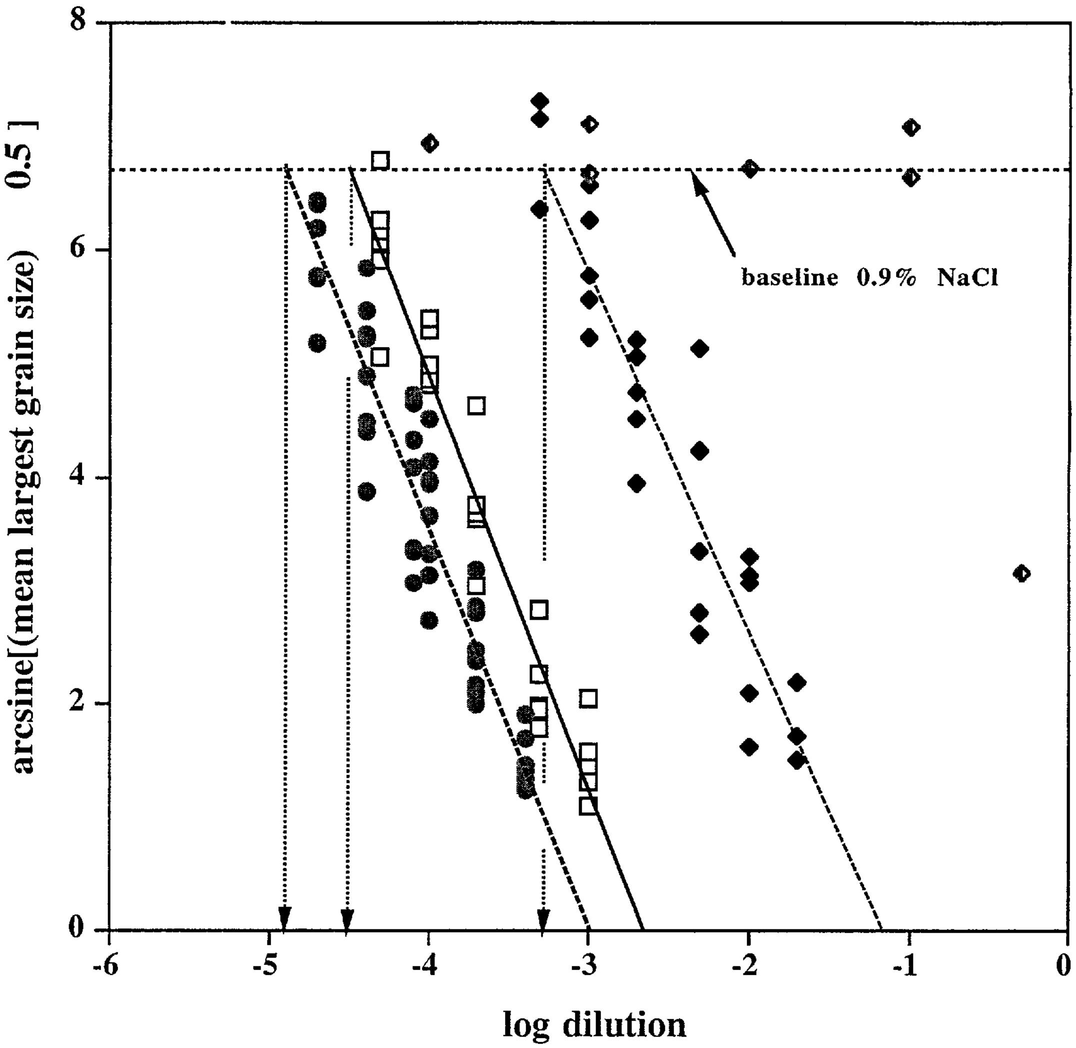

arcsine[(mean largest grain size) 0.5]
8
6
4
2
0
-6
-5
-4
-3
-2
-1
0
log dilution

FIG. 8.23



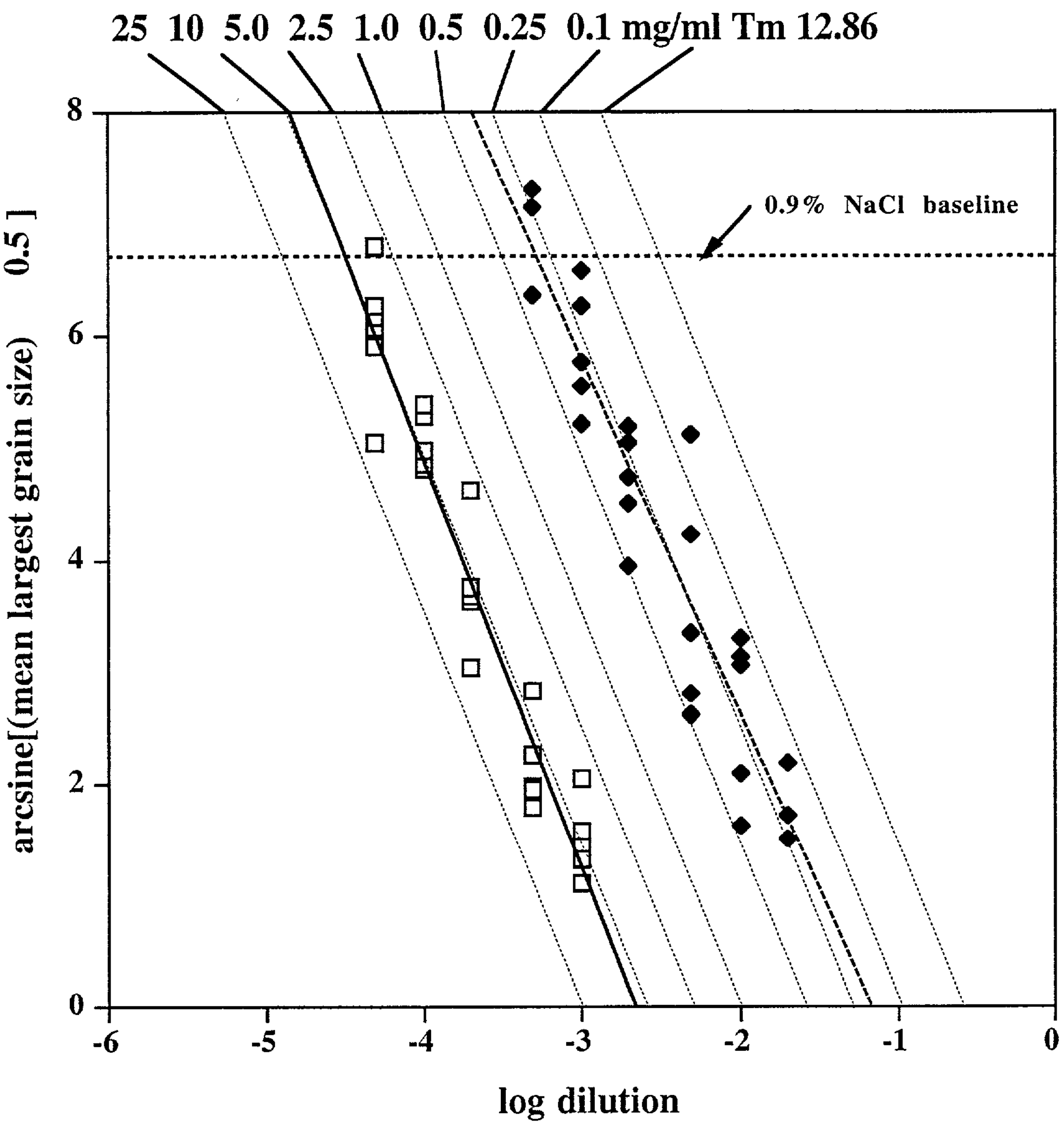
25
10
5.0
2.5
1.0
0.5
0.25
0.1 mg/ml Tm 12.86
baseline 0.9% NaCl
arcsine[(mean largest grain size) 0.5]
log dilution
8
6
4
2
0
-6
-5
-4
-3
-2
-1
0

FIG. 8.24



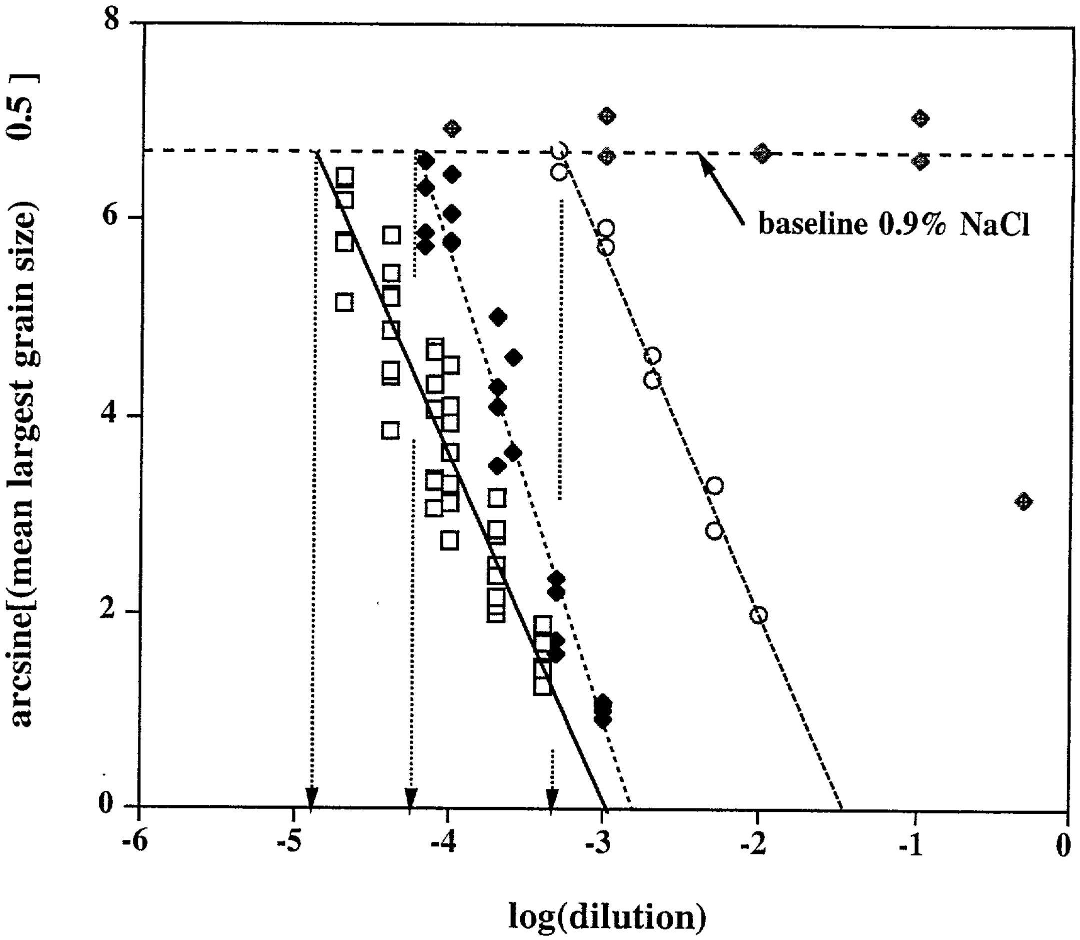
8
6
4
2
0
arcsine[(mean largest grain size) 0.5]
baseline 0.9% NaCl
-6
-5
-4
-3
-2
-1
0
log dilution

FIG. 8.25



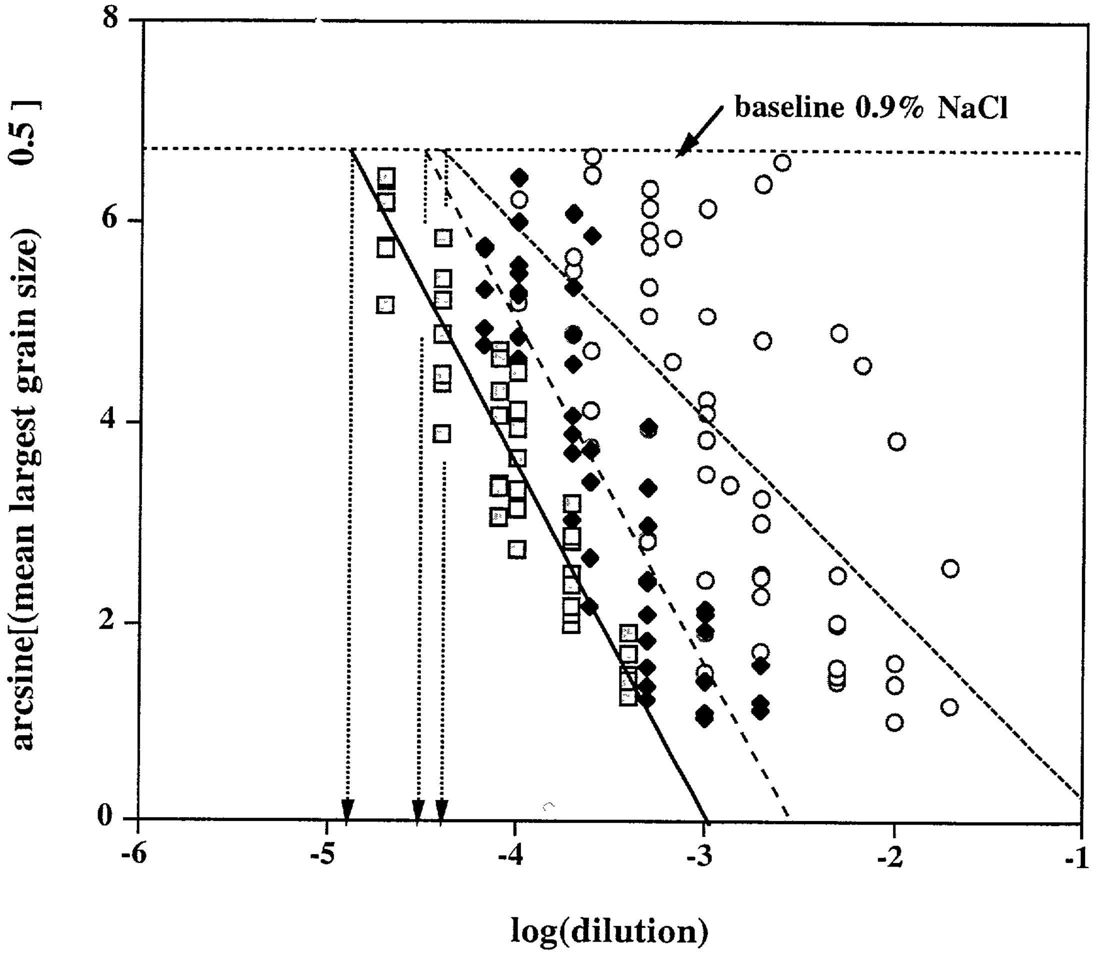
25 10 5.0 2.5 1.0 0.5 0.25 0.1 mg/ml Tm 12.86
0.9% NaCl baseline
arcsine[(mean largest grain size) 0.5]
log dilution
8
6
4
2
0
-6
-5
-4
-3
-2
-1
0

FIG. 8.26



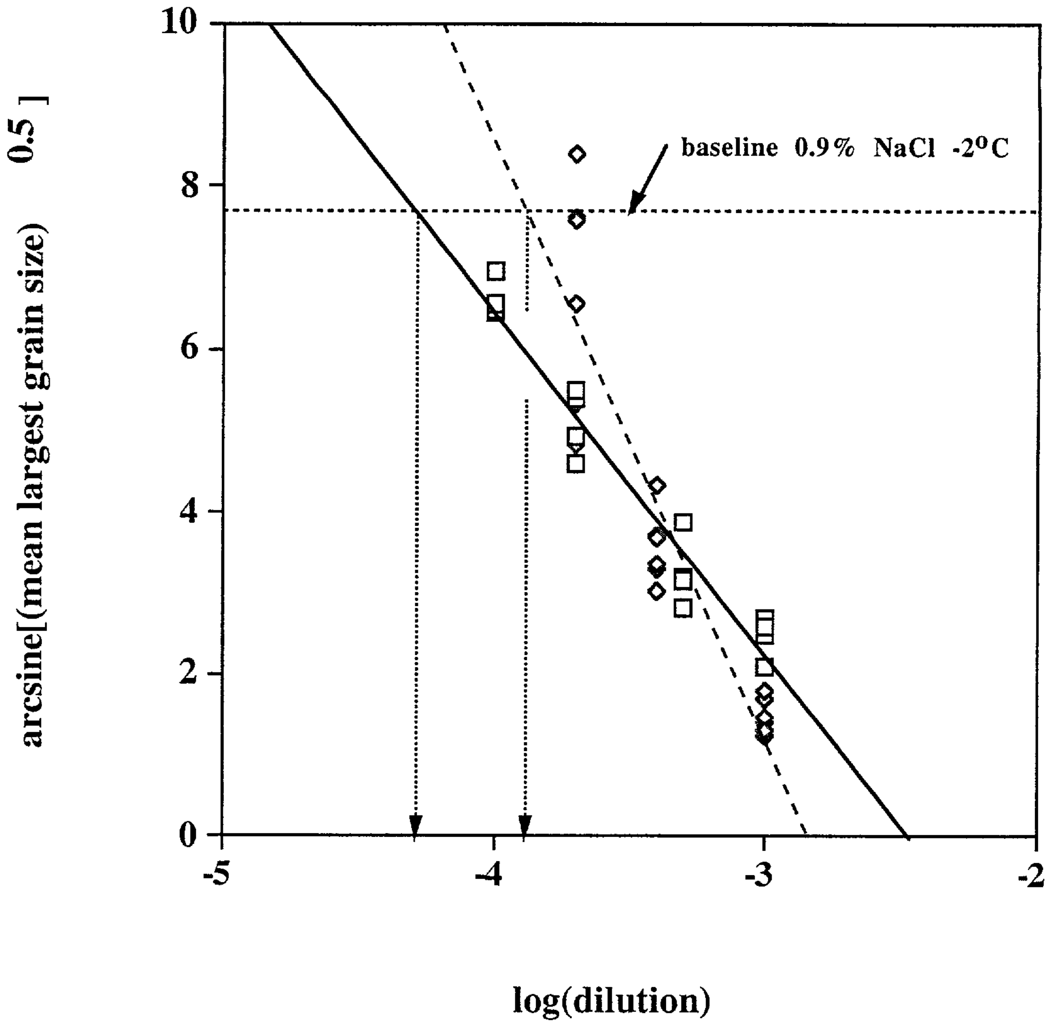
arcsine[(mean largest grain size) 0.5]
log(dilution)
baseline 0.9% NaCl
8
6
4
2
0
-6
-5
-4
-3
-2
-1
0

FIG. 8.27



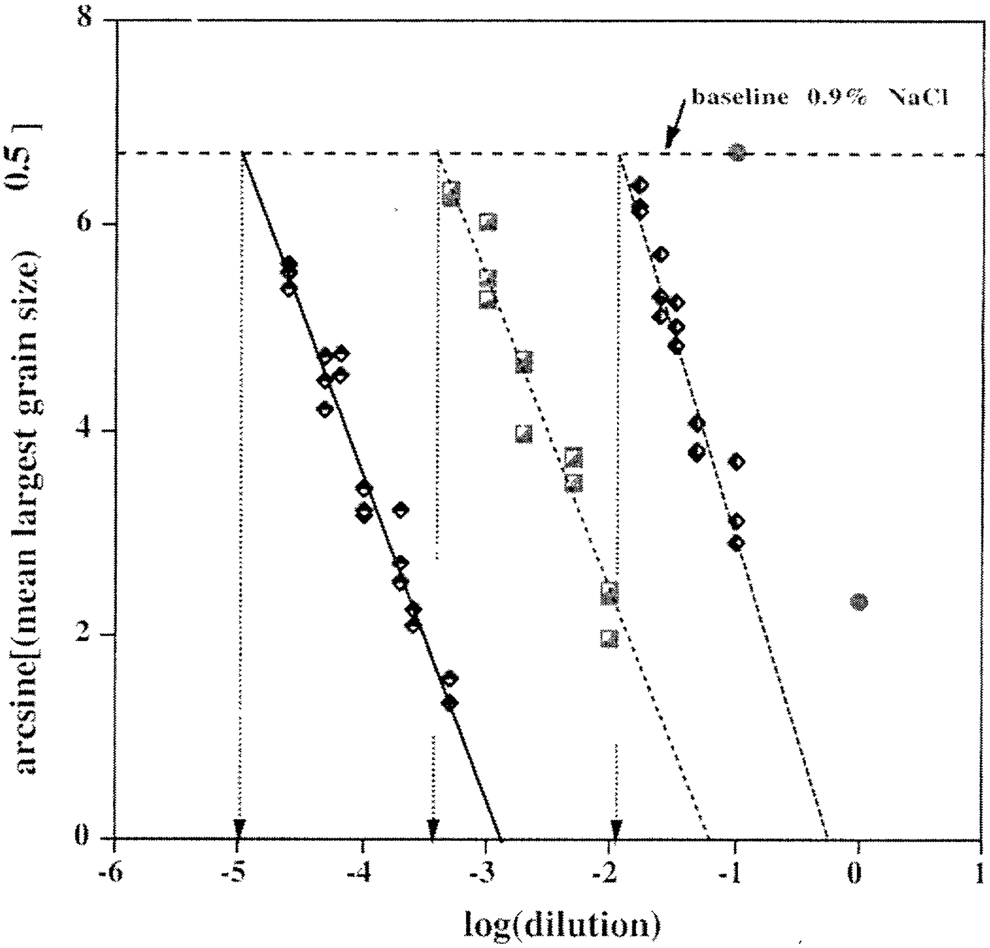
8
6
4
2
0
-6
-5
-4
-3
-2
-1
baseline 0.9% NaCl
arcsine[(mean largest grain size) 0.5]
log(dilution)

FIG. 8.28



baseline 0.9% NaCl -2°C
10
8
6
4
2
0
-5
-4
-3
-2
arcsine[(mean largest grain size) 0.5]
log(dilution)

FIG. 8.29



baseline 0.9% NaCl
arcsine[(mean largest grain size) 0.5]
log(dilution)
8
6
4
2
0
-6
-5
-4
-3
-2
-1
0
1

FIG. 8.30

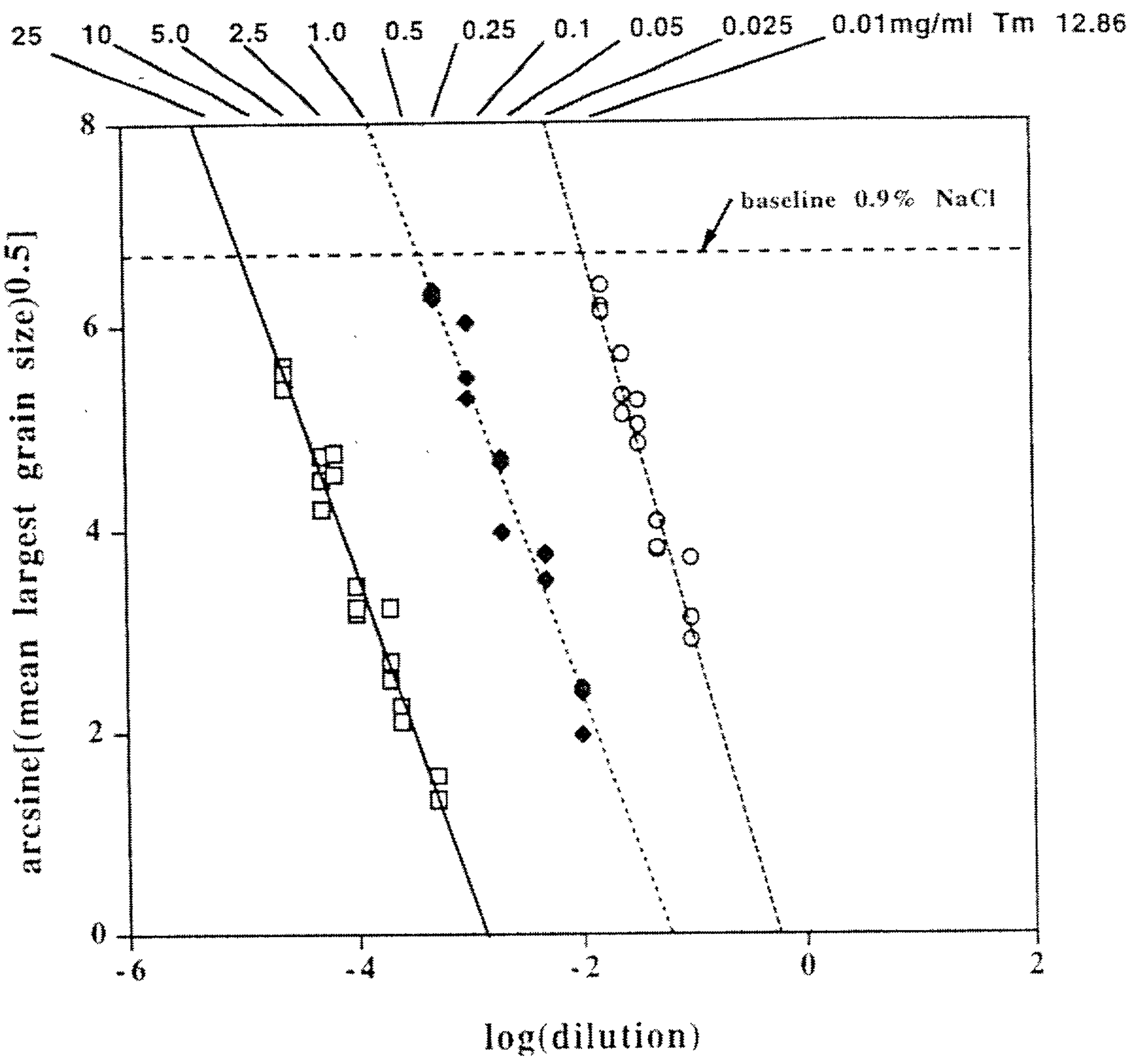
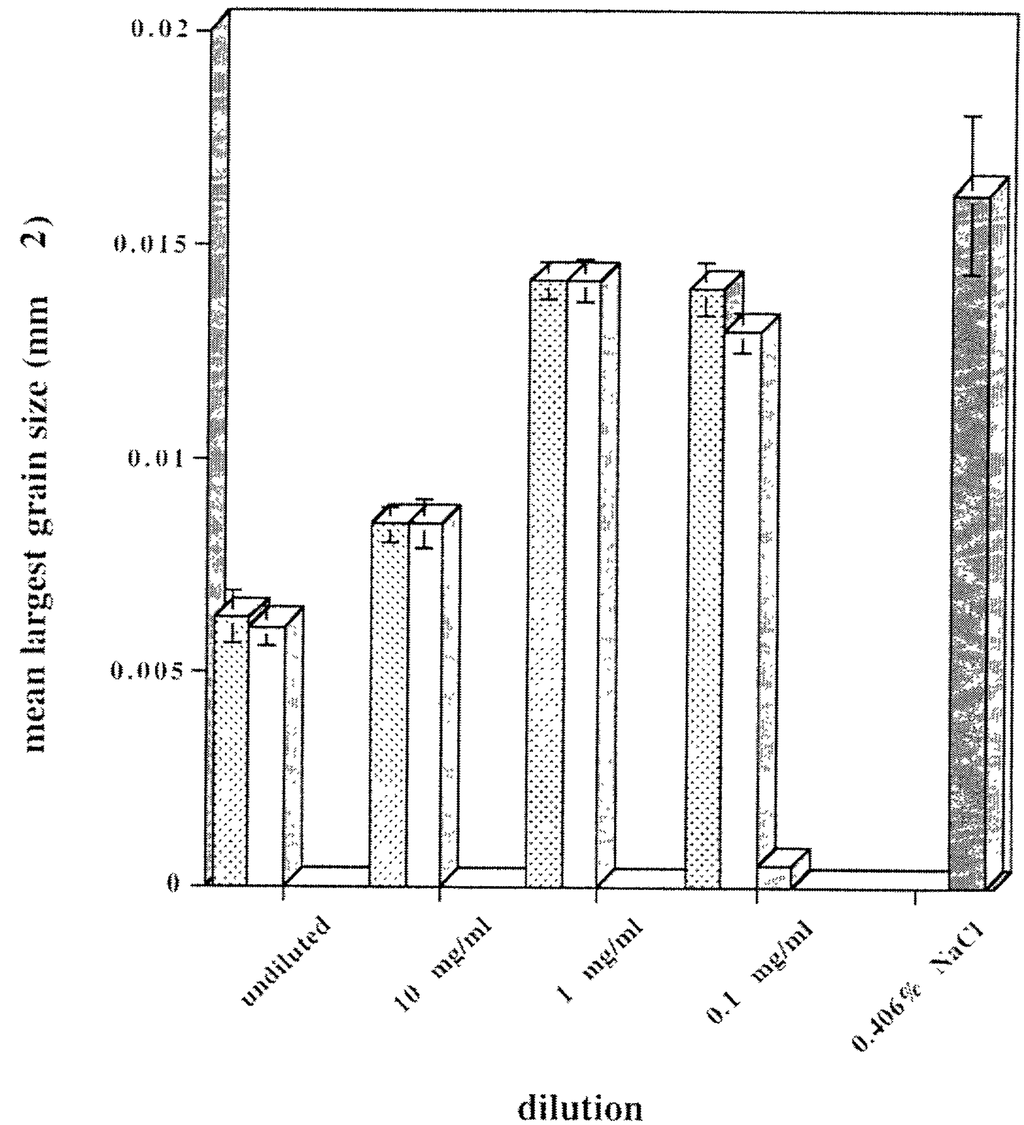

25
10
5.0
2.5
1.0
0.5
0.25
0.1
0.05
0.025
0.01mg/ml Tm 12.86
baseline 0.9% NaCl
arcsine[(mean largest grain size)0.5]
8
6
4
2
0
-6
-4
-2
0
2
log(dilution)

FIG. 8.31



mean largest grain size (mm 2)
0.02
0.015
0.01
0.005
0
undiluted
10 mg/ml
1 mg/ml
0.1 mg/ml
0.406% NaCl
dilution

FIG. 8.32

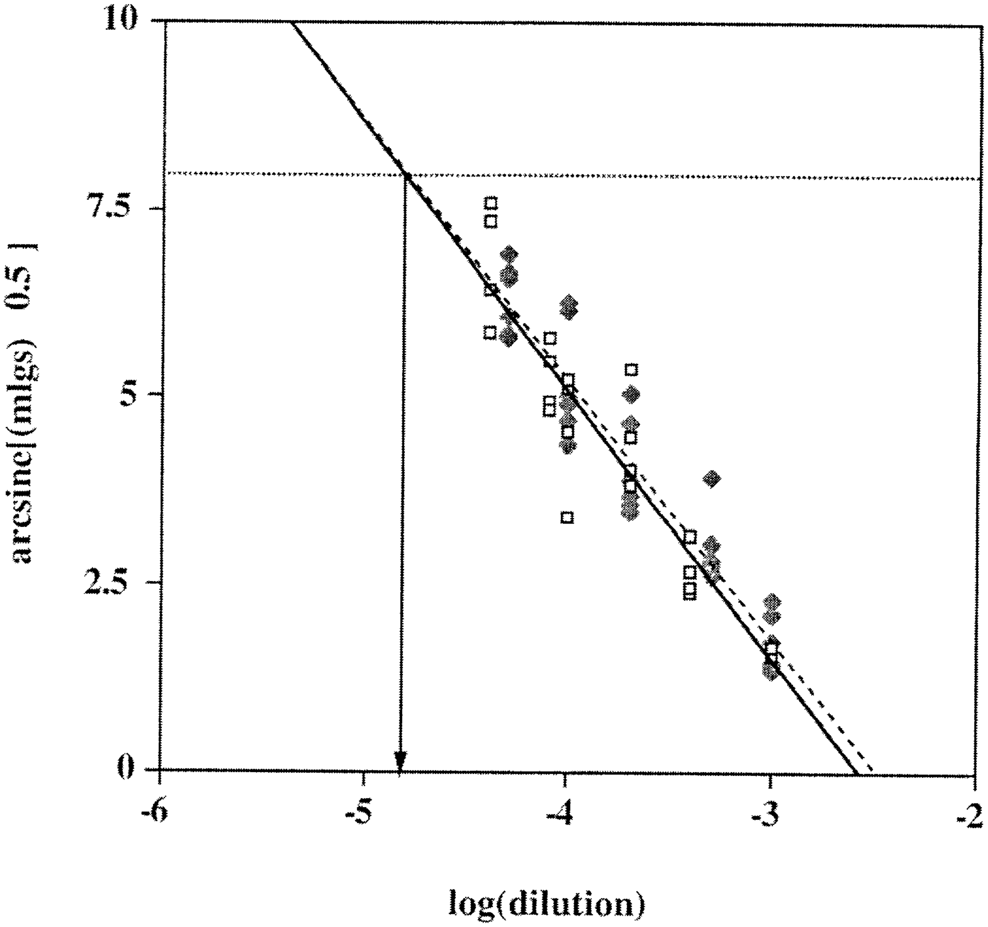
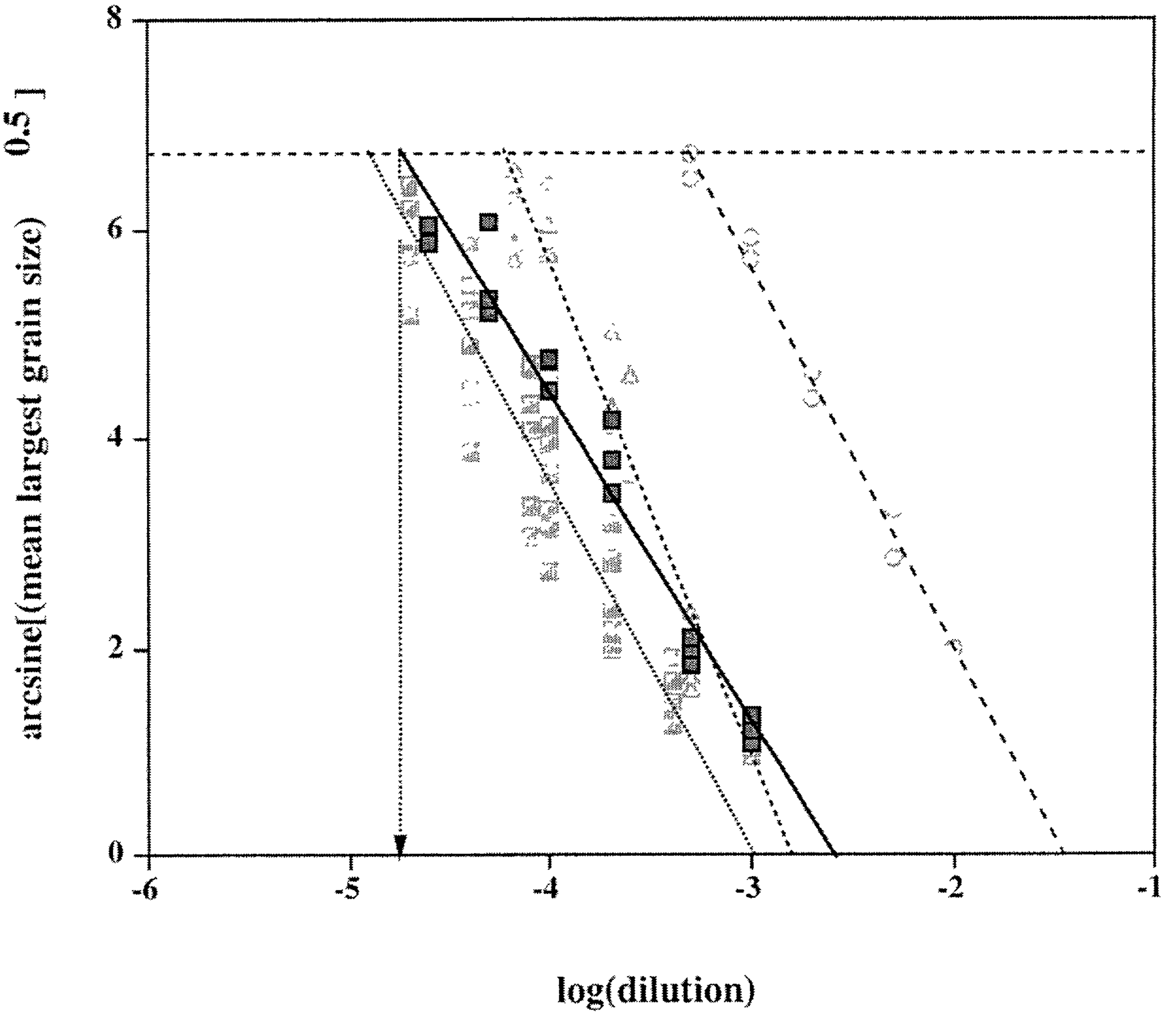


FIG. 8.33



arcsine[(mean largest grain size) 0.5]
8
6
4
2
0
-6
-5
-4
-3
-2
-1
log(dilution)

FIG. 8.34

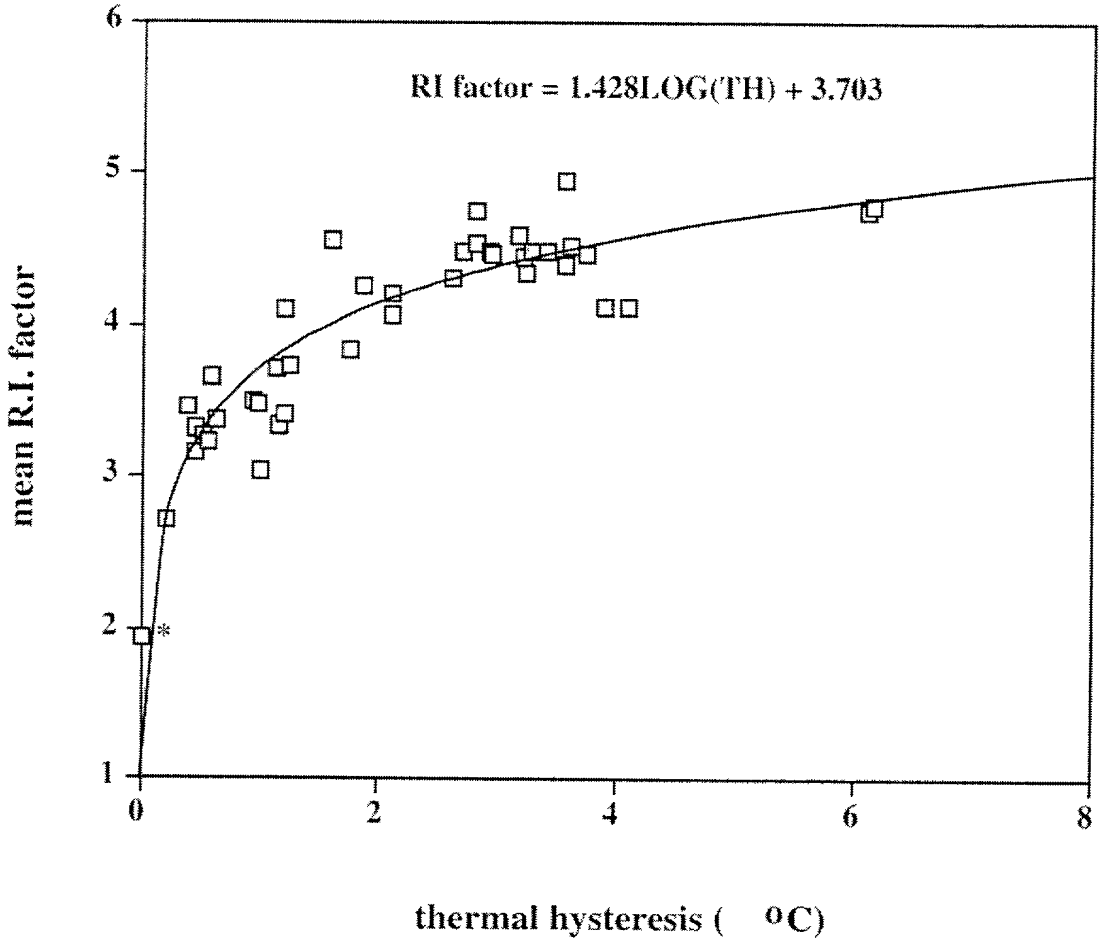

RI factor = 1.428LOG(TH) + 3.703
mean R.I. factor
thermal hysteresis (°C)
6
5
4
3
2
1
0
2
4
6
8
*

FIG. 8.35

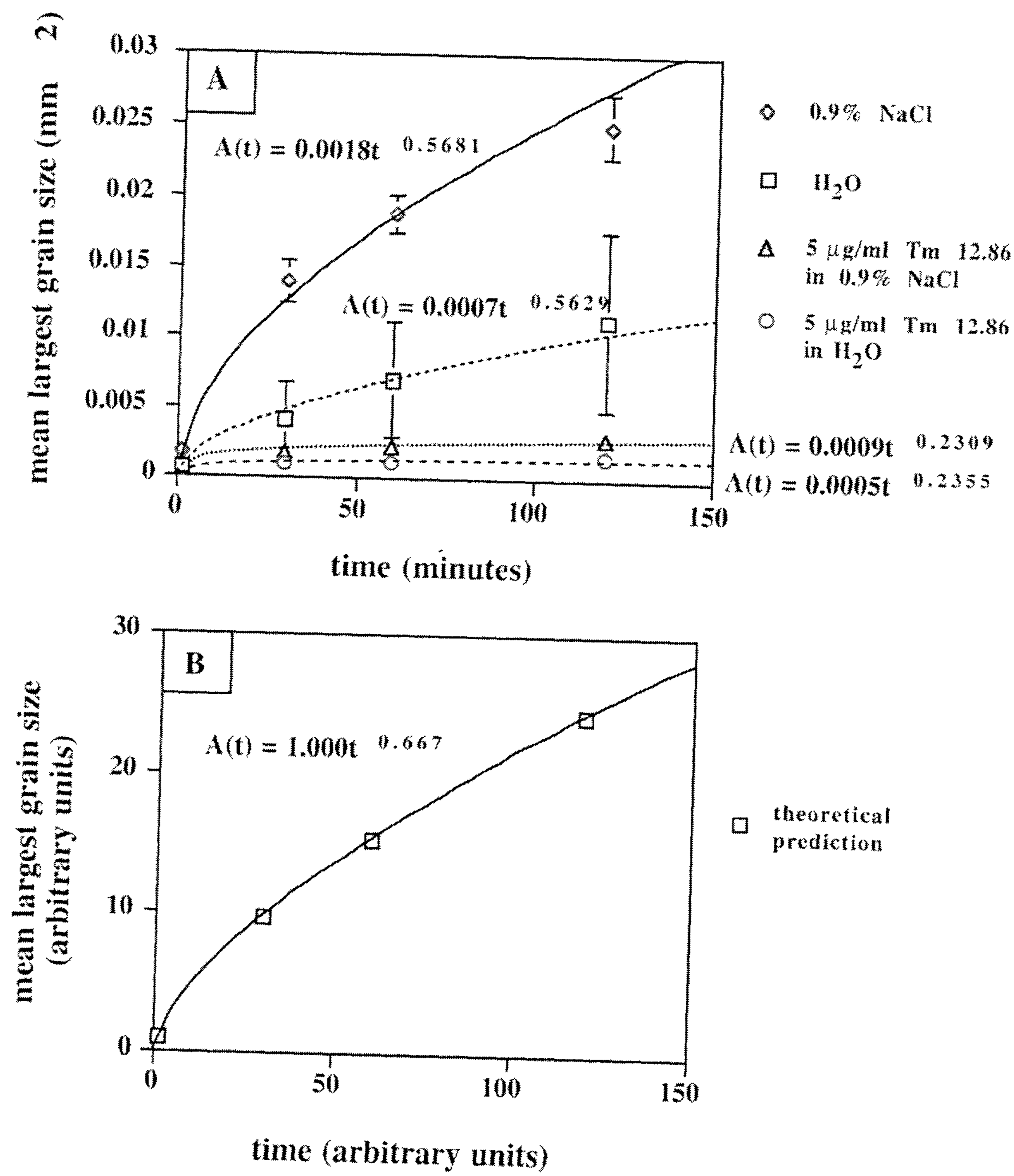

A
mean largest grain size (mm 2)
0.03
0.025
0.02
0.015
0.01
0.005
0
A(t) = 0.0018t 0.5681
A(t) = 0.0007t 0.5629
A(t) = 0.0009t 0.2309
A(t) = 0.0005t 0.2355
0.9% NaCl
H2O
5 µg/ml Tm 12.86 in 0.9% NaCl
5 µg/ml Tm 12.86 in H2O
0
50
100
150
time (minutes)
B
mean largest grain size (arbitrary units)
30
20
10
0
A(t) = 1.000t 0.667
theoretical prediction
0
50
100
150
time (arbitrary units)

FIG. 8.36

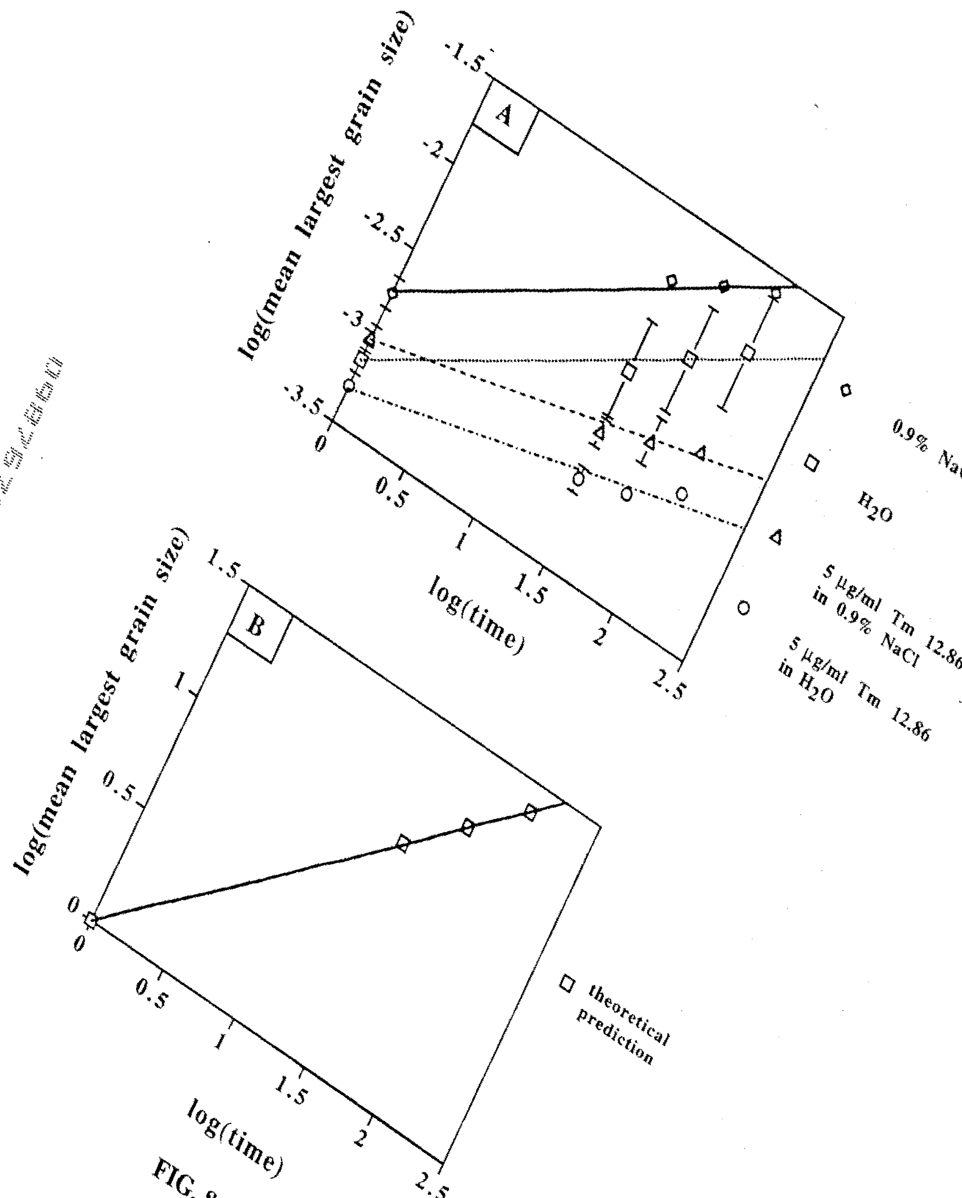
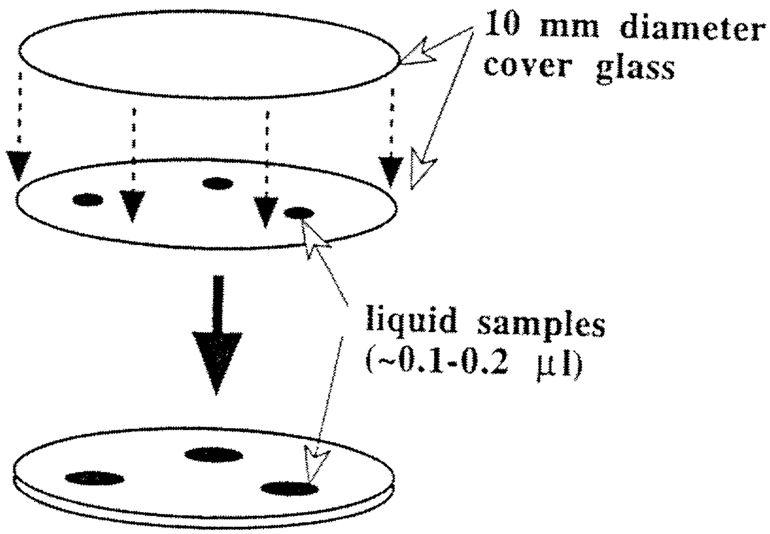

A
log(mean largest grain size)
-1.5
-2
-2.5
-3
-3.5
0
0.5
1
1.5
2
2.5
log(time)
0.9% NaCl
H2O
5 µg/ml Tm 12.86 in 0.9% NaCl
5 µg/ml Tm 12.86 in H2O
B
log(mean largest grain size)
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
log(time)
theoretical prediction

FIG. 8.37

"Sandwich" method of R.I. assessment

1.



2.

3. FREEZE ON ~-80 C
ALUMINUM PLATE (~10 MIN.)

4. PLACE ON COLD STAGE,
ANNEAL AT -6 C UP TO
12+ HOURS

FIG. 8.38

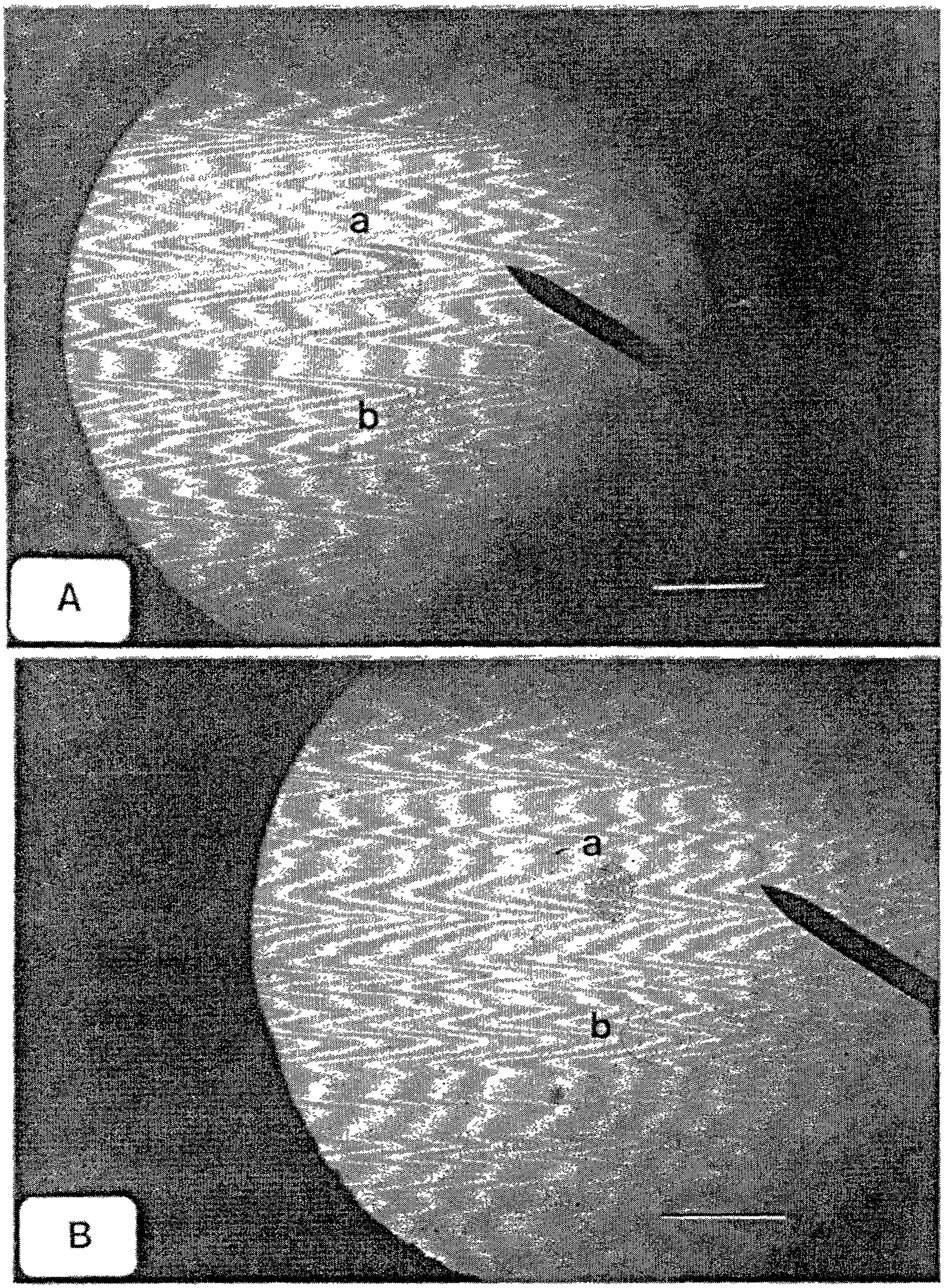


FIG. 8.39

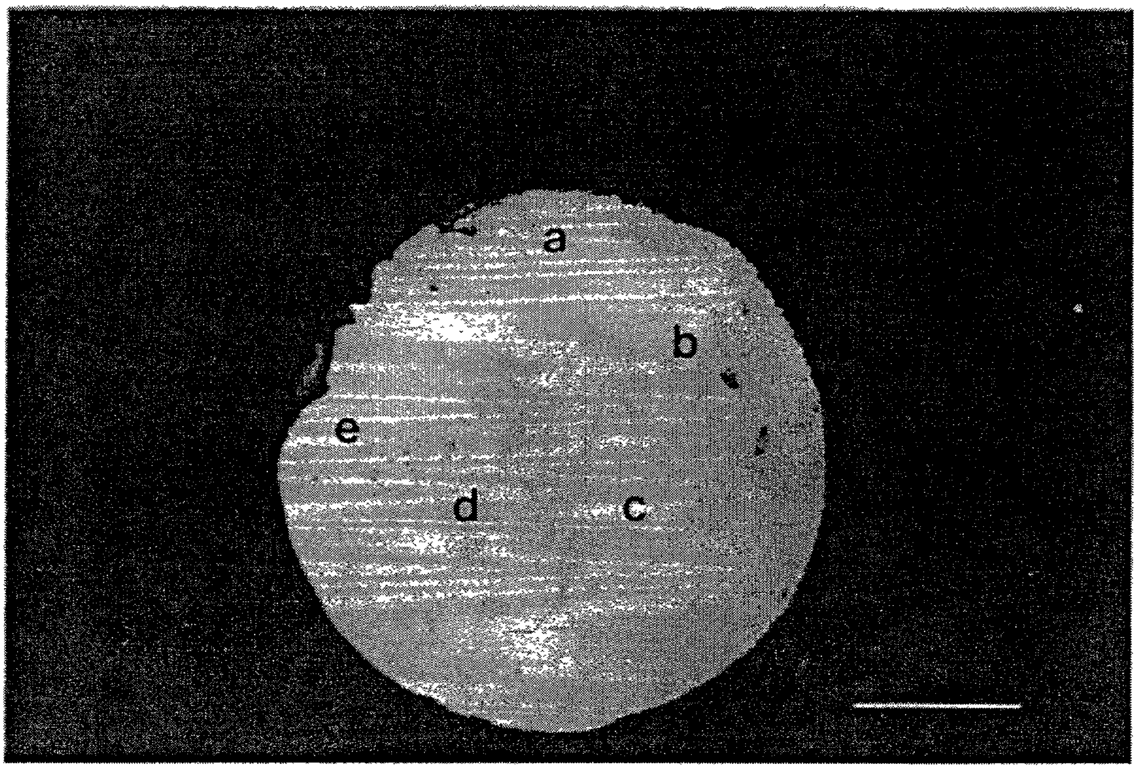


FIG. 8.40

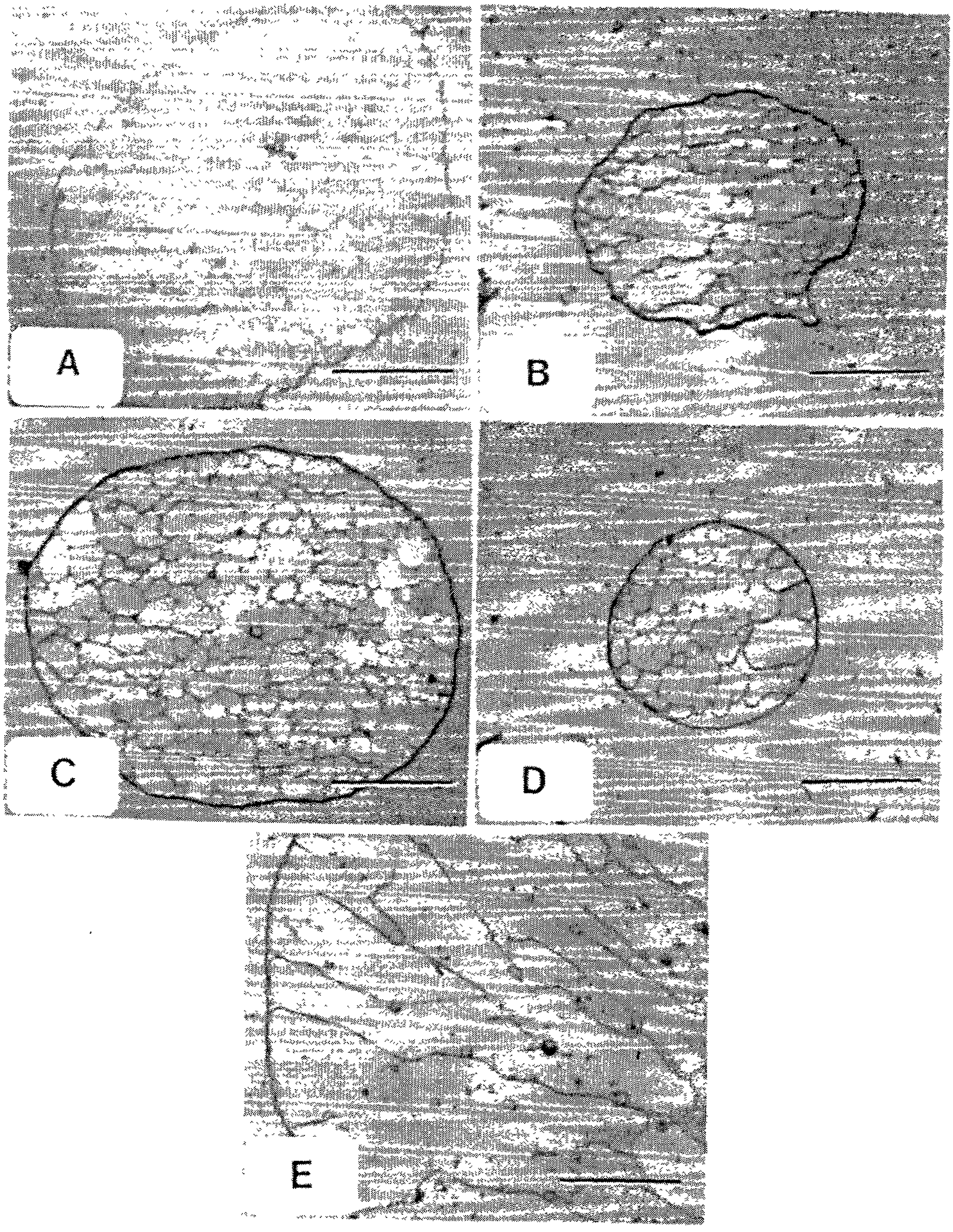


FIG. 8.41

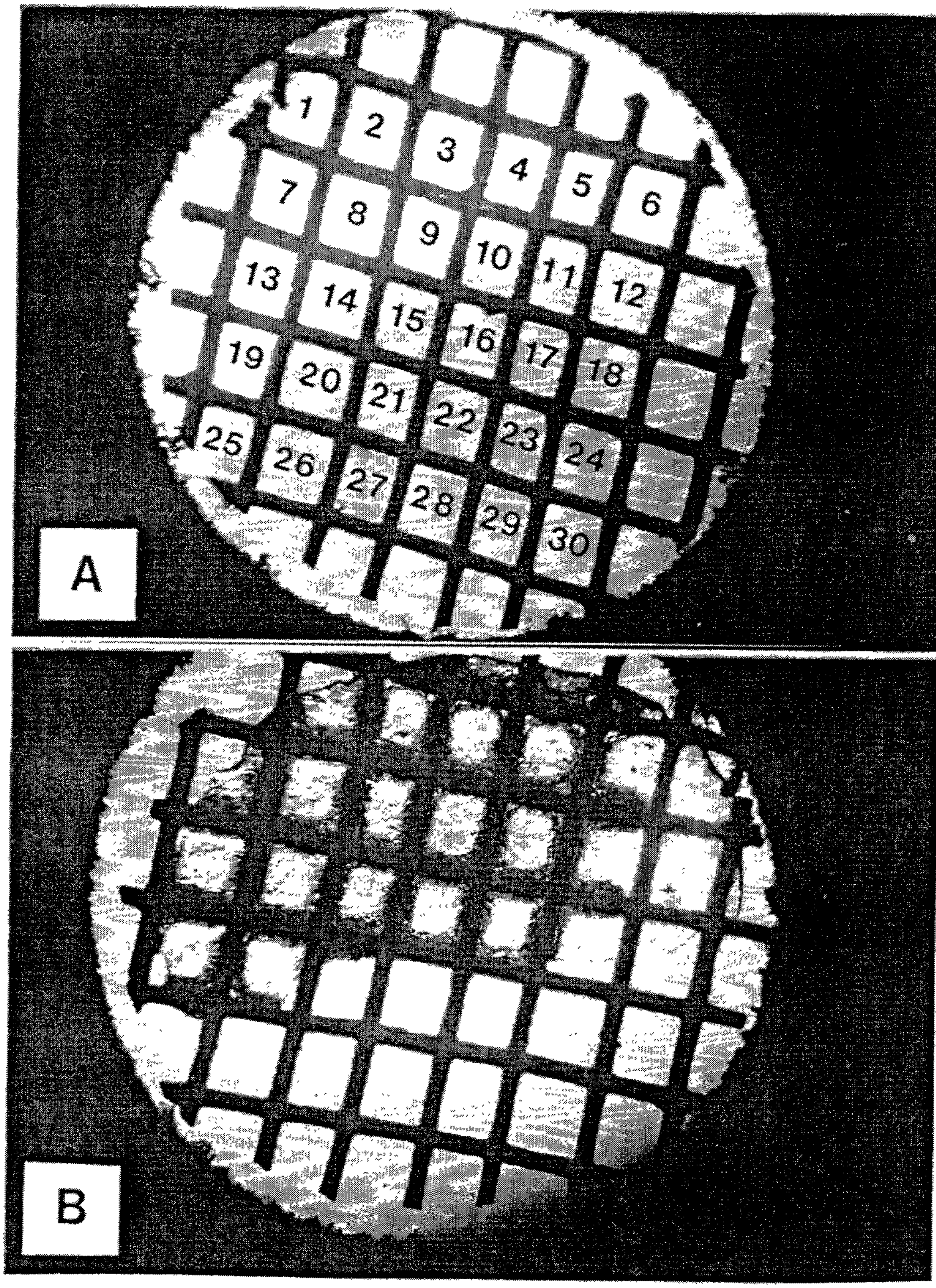

A
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
B

FIG. 8.42

DNA sequence of Tm 13.17 cDNA clone

```
          B         E
          a         c
          m         o
          H         R
          I         I
  1 AGTGGATCCAAAGAATTCGGCACGAGACTACTAAGATGAAGTTGCTCTGTTGTCTAATCT
                                        M  K  L  L  C  C  L  I  S

 61 CCCTCATTCTGTTGGTCACAGTTCAGGCCCTGACCGAGGCACAAATTGAGAAACTGAACA
     L  I  L  L  V  T  V  Q  A  L  T  E  A  Q  I  E  K  L  N  K

121 AGATCAGCAAAAAATGTCAAAATGAAAGTGGAGTGTCGCAAGAGATCATAACCAAAGCTC
     I  S  K  K  C  Q  N  E  S  G  V  S  Q  E  I  I  T  K  A  R

181 GCAACGGTGACTGGGAGGACGATCCTAAACTGAAACGCCAAGTTTTTTGCGTGGCCAGGA
     N  G  D  W  E  D  D  P  K  L  K  R  Q  V  F  C  V  A  R  N

241 ACGCCGGTCTGGCCACGGAATCGGGAGAGGTGGTGGTCGACGTGTTGAGGGAGAAGGTGA
     A  G  L  A  T  E  S  G  E  V  V  V  D  V  L  R  E  K  V  R

301 GGAAGGTCACTGACAACGACGAAGAAACTGAGAAAATCATCAATAAGTGCGCCGTCAAGA
     K  V  T  D  N  D  E  E  T  E  K  I  I  N  K  C  A  V  K  R

361 GAGATACTGTTGAAGAGACGGTGTTCAATACTTTCAAATGTGTCATGAAAAACAAGCCAA
     D  T  V  E  E  T  V  F  N  T  F  K  C  V  M  K  N  K  P  K

421 AGTTCTCACCAGTTGATTGAACCACCACGACTAGTAGATGGTTCAAATGGTGTGCTTTAC
     F  S  P  V  D  *
                                                            X
                                                            h
                                                            o
                                                            I
481 ATATAAAAATAAAGTGTTTCTGATGTAAAAAAAAAAAAAAAAAAAAAAAAAACTC
            polyadenylation signal  poly (A) tail (26)

537 AGAGTATTCTAGAGCGGCCGCGGGCCCATCGTTTTCCACCC
```

FIG. 8.43

```
1    GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCGTTCGCCGCC
                  M  K  L  L  L  C  F  A  F  A  A

47   ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA
     I  V  I  G  A  Q  A  L  T  D  E  Q  I  Q  K

92   AGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGTGTCC
     R  N  K  I  S  K  E  C  Q  Q  V  S  G  V  S

137  CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGATGAT
     Q  E  T  I  D  K  V  R  T  G  V  L  V  D  D

182  CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGA
     P  K  M  K  K  H  V  L  C  F  S  K  K  T  G

226  GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
     V  A  T  E  A  G  D  T  N  V  E  V  L  K  A

271  AAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTG
     K  L  K  H  V  A  S  D  E  E  V  D  K  I  V

316  CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
     Q  K  C  V  V  K  K  A  T  P  E  E  T  A  Y

361  GACACCTTCAAGTGTATTTACGACAGCAAACCTGATTTCTCTCCT
     D  T  F  K  C  I  Y  D  S  K  P  D  F  S  P

406  ATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT
     I  D  *

                                polyadenylation signal

451  ACTATCGTTATGTAAAAAAAAAAAAAAAAAA
```

poly (A) tail

FIG. 8.44